Electrophysiology of the Heart

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WITH A FOREWORD BY *Franklin D Johnston* M D

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To
Joseph Erlanger
J A E Eyster
and
Walter J Meek

ELECTROPHYSIOLOGY OF THE HEART

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FOREWORD

I consider it quite a privilege and an honor to be asked to write the foreword for this book by Brian Hoffman and Paul Cranefield My qualifications for this task may properly be questioned since I have had no personal experience with the exacting technique required for the registration of the transmembrane potentials from single fibers of heart muscle and this book is almost entirely concerned with presentation and discussion of records obtained by this method Perhaps however, my long experience in clinical and experimental electrocardiography may entitle me to express an opinion about work like the authors are doing with microelectrodes

The action potentials recorded by a microelectrode placed within single cells in the muscle anywhere in the heart may be regarded as the basic sources from which the electrocardiogram recorded by any type of surface lead arises The situation in the heart is extremely complicated because so many cells having action potentials of different duration and form are involved Nevertheless a clear understanding of the different kinds of potentials that exist normally in the SA and AV nodes in the His bundle and interconnected special conducting pathways, and in ordinary atrial and ventricular muscle fibers together with accurate information regarding the effects that many factors (changes in rate temperature electrolytes oxygen supply or presence of physiologic agents like acetylcholine or ephinephrine and drugs like digitalis or quinidine) have on these action potentials is essential before many features of normal and abnormal electrocardiograms can be further clarified

One of the fields of greatest ignorance in clinical electrocardiography concerns the T waves Why do some apparently healthy individuals have flat or inverted T waves in leads where these waves are 'normally upright? To put the question in even more fundamental form why do subjects with normal hearts have upright

T waves in leads I, II, and in the left precordial leads, where the chief deflections of the QRS complexes are also upright? Dr F N Wilson provided a partial answer to this question many years ago by pointing out the existence of the "ventricular gradient" The complete answer, however, must lie in differences in the duration or form of transmembrane action potentials during repolarization, especially in phases 2 and 3, that exist normally in ventricular muscle

The problem of the T waves is only one of many puzzles that may eventually be solved by the systematic use of techniques for registration of action potentials from single fibers in the heart Much light has already been thrown on the reasons why cells in the SA node and occasionally elsewhere serve as pacemakers, and information pertaining to AV conduction and excitability of various types of heart muscle fibers which may prove to be the key to the genesis of many of the cardiac arrhythmias is rapidly being collected Need more be said to emphasize the potential value and importance of the matters discussed in this book?

In Chapter 1 the reader will find a discussion of the technique for obtaining action potentials from single cardiac fibers a survey of the different types of records to be found in various kinds of fibers and some discussion of the relationships between these records and those obtained by external leads Chapter 2 is devoted to the electrical properties of excitable cells and a summary of the facts known regarding the ionic basis for the resting membrane potential and the action potential Chapters 3 4, 5, 6 and 7 are concerned with detailed discussions of the action potentials obtained from muscle cells in the atrium ventricle sinoatrial node atrioventricular node, and Purkinje fibers respectively These chapters not only point out the variations in the records found normally in different species but summarize available data concerning the effects of many factors such as variation in rate or temperature changes in sodium or potassium content of external medium or presence of acetylcholine or epinephrine on the action potentials obtained from the various cells Although much of the material presented (some of it previously unpublished) was obtained by the authors or by others in their laboratories a great deal of work done by others is presented and discussed When differences in results or interpretations have appeared, these discrepancies or divergent opinions have been

clearly stated and the need for further and perhaps better studies to settle the questions involved has been pointed out I was greatly pleased to see that the authors have not failed to appreciate that some of the conclusions arising from recent work by themselves and others had been predicted from much earlier studies by that great physiologist Joseph Erlanger (see Chap 6)

Chapter 8 is devoted to a discussion of the excitability of heart muscle cells using both cathodal and anodal stimuli The results of these studies are very interesting particularly those obtained by anodal stimuli, since they appear to have direct bearing on the "supernormal phase" of conduction occasionally seen in humans in high grade AV heart block and in other arrhythmias Much of Chapter 9 is concerned with summary and electrophysiologic interpretation of material presented earlier in the book This procedure is excellent and helps greatly to emphasize and fix in the reader s mind many of the important matters presented previously This chapter concludes with a discussion of a possible ionic mechanism for repolarization I am not in a position to say more than that this proposal seems to be a logical and reasonable one

This book summarizes the important information available and adds much new material in this very interesting and rapidly developing field It should be of great value to physiologists or physicians seriously interested in electrophysiology

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PREFACE

The electrocardiographic record of the electrical activity of the whole heart depends upon the shape of the action potentials of the various cells of the heart and upon the sequence of activation of those cells. The development of the method of microelectrode recording has made possible a new degree of precision in our knowledge of the action potentials of single cardiac cells. The technique of single-cell recording is only ten years old but a great deal of work has been done on the heart in that time. It is our hope that the presentation of the results of that work which we have undertaken to give in this book will be of use not only to physiologists but also to those interested in electrocardiography. We have also touched very briefly upon results obtained by new methods of studying the sequence of activation of the heart. It is not too much to hope that in the next five or ten years new studies of the sequence of activation will be combined with the results of single cell studies to provide electrocardiography with a more systematic foundation in experimental electrophysiology than it has previously had.

We are grateful to all our friends and colleagues who have helped and encouraged us in this work. Our indebtedness to the men to whom this book is dedicated is twofold. We have found their published papers valuable and stimulating and one of us (P. F. C.) studied for some years with Dr. Eyster and Dr. Meek. We have had many valuable discussions with Dr. Silvio Weidmann of Bern and with Dr. Antonio Paes de Carvalho of Rio de Janeiro, each of whom has also been most generous about sending us unpublished material. We have also benefited greatly from our association with Dr. Kojiro Matsuda, Dr. Walmor Carlos de Mello, Dr. Morris Kleinfeld, Dr. Jackson Stuckey and Dr. John J. Kelly, all of whom either have helped us in our research or have assisted us by discussion and by supplying unpublished information.

We both owe our interest in cardiac excitability to the studies of that subject which were initiated in the Department of Physiology of the State University of New York, Downstate Medical Center by Drs Oscar Orias and C McC Brooks We are further indebted to Dr Brooks for his general encouragement of our studies of cardiac electrophysiology

Much previously unpublished research from our laboratory is reported in this book That research was supported by grants from The American Heart Association The New York Heart Association, and The National Heart Institute of the United States Public Health Service (U S P H Grant H-3916) The manuscript was completed and submitted to the publisher on July 15 1959

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Circulation Research, 7 19–23, 1959 Figures 6 1, 6-3, 6 4 and 6 5 are reprinted by permission from Figs 1, 2 3 and 5 respectively of Electrical Activity of Single Fibers of the Atrioventricular Node by B F Hoffman, A Paes de Carvalho, W Carlos de Mello, and P F Cranefield *Circulation Research*, 7 11–19, 1959 Figure 6-22 is reprinted by permission from Fig 1 of Physiologic Evidence for a Dual A-V Transmission System by G K Moe, J B Preston, and H Burlington, *Circulation Research*, 4 357–375, 1956 The figures from *Circulation Research* are reprinted by permission of Grune & Stratton, Inc We should like to express our appreciation to the publishers mentioned above for permission to reprint illustrations, we should also like to express our appreciation to the editors of the journals and to the authors of the articles for their permission to reprint figures

CONTENTS

1

RECORDING TECHNIQUES

Three quite different techniques are used to record electrical activity directly from cardiac muscle. One employs conventional unipolar or bipolar surface electrodes in close proximity to uninjured tissue, the second employs one lead in contact with injured muscle and another which may be either close to or distant from the site of injury. In the third and most recent technique one intracellular microelectrode is paired with an extracellular lead to record the transmembrane potential of a single cardiac fiber. Although we have attempted to treat most of the subject matter of this book in terms of results obtained by means of the intracellular microelectrode, certain important aspects of cardiac electrophysiology have as yet been studied only by other methods. This chapter describes those methods and attempts to show which of them is appropriate in the study of certain problems.

TRANSMEMBRANE POTENTIALS OF SINGLE CARDIAC FIBERS

Recording the transmembrane potential of single fibers by means of an intracellular microelectrode provides an accurate and sensitive index of the electrical changes associated with activity of excitable tissues. One limitation of this method is that only local changes in the single cell or part of a cell can be detected with a single electrode. Moreover, the delicate glass microelectrode is easily broken or dislodged from the cell under study. Many special precautions are necessary in terms of the properties of amplifiers and recording equipment in order to avoid artifactual distortion of the records

However, this technique permits the most accurate determination of the magnitude and time course of the transmembrane action potential and also succeeds where other methods fail, in demonstrating the peculiarities of the excitatory process in the sinoatrial and atrioventricular node An understanding of microelectrode methods is thus essential for any comprehension of modern cardiac electrophysiology

Microelectrode Methods

It is possible to record the electrical activity of a single cell or of a single unit of the cardiac syncytium by using a microelectrode of the type introduced by Ling and Gerard (1949) Such a microelectrode is a fine glass capillary pulled to a tip diameter of less than 1 μ and filled with a concentrated electrolyte The electrolyte acts as a conductor which is insulated except at the open tip, by the surrounding glass capillary The outside diameter of the microelectrode tip is critical Ling and Gerard showed that in order to record the transmembrane potential of single fibers of frog sartorius muscle the tip of the electrode must be 1 μ or less in diameter, larger electrodes damaged the fiber membrane and gave erroneously low values Somewhat similar estimates of size were obtained by Woodbury, Hecht and Christopherson (1951) for frog heart and by Nastuk and Hodgkin (1950) for frog sartorius In general these estimates of the critical size can be accepted To record potentials from certain very small cells such as the fibers of the sinoatrial node and parts of the atrioventricular node or from small nerve fibers and cell bodies it is necessary to employ electrodes that are considerably less than ½ μ in diameter For studies of large cells such as the giant axon of squid it is likely that a somewhat larger electrode tip is permissible

A number of different substances have been employed to fill microelectrodes Concentrated potassium chloride is used most frequently, although other electrolytes and even metals are sometimes substituted (see Shanes 1958) Theoretical considerations of the generation of junction potentials at the electrode tip as well as the results of actual experimentation (Nastuk and Hodgkin, 1950, Adrian 1956 Shanes 1958) have led most workers to use a 3 molar solution of KCl Filling is most easily accomplished by boiling the electrodes held on some suitable mount, until all the

air is replaced by the KCl, other techniques involve filling in a vacuum filling first with alcohol and then KCl and filling by capillarity In our hands the first of these methods has proved simple and reliable for work in which the microelectrode is employed to record the transmembrane potential When the electrode is used for other purposes the composition of the electrolyte used for filling will differ Thus for studies of the pharmacology of the postsynaptic membrane of the myoneural junction electrodes may be filled with acetylcholine curare, or other substances If a current of the appropriate polarity is passed through the electrode, calculable quantities of the electrolyte can be ejected from the tip (Nastuk 1953) This technique can be used when the electrode is either extracellular or intracellular in position, in the latter case however the nature of the ionic species which carry current across the cell membrane is unknown and this factor must be considered when the purpose of the injection is an alteration of the ionic composition of the intracellular fluid

The electrode is commonly mounted directly on a micromanipulator and the electrode tip is introduced into the cell during direct observation through a microscope If the tissue under study moves vigorously this method may fail in which case it is possible to suspend a short microelectrode from a very fine flexible wire (Woodbury and Brady 1956), the electrode tip is lowered against the surface and allowed to penetrate under the influence of the tissue movement Although this latter method has many disadvantages it does permit records to be obtained from moving tissue for long periods of time

Use of the intracellular microelectrode to record the transmembrane potential of a single fiber requires special input circuits amplifiers and display apparatus These have recently been reviewed (Grundfest 1957) and will be mentioned only in summary Because of the high electrical resistance of the microelectrode (5 to 50 megohms) and the requirement for minimal grid current a cathode follower input is employed Also since the large electrode resistance and relatively high capacity tend to give a poor response to high frequency components of the signal it is desirable to employ some form of input capacity neutralization (Amatniek, 1958) Direct coupled amplifiers of extremely low drift are necessary if measurements of the resting potential over a prolonged period of

time are to be meaningful Finally, the recording device should have a frequency response capable of following transients up to 30 kc, standard electrocardiograph machines are thus of little value in giving a faithful record of either the rising phase or amplitude of the transmembrane action potential

The Contour of the Cardiac Action Potential

The Resting Potential If a microelectrode is advanced slowly through several layers of cardiac cells sudden sharp shifts of potential are recorded During these shifts the microelectrode records either no difference of potential or becomes 80 to 90 mv negative with respect to the external reference electrode These potential changes are thought to represent the appearance and disappearance of the transmembrane resting potential as the electrode enters and leaves individual cells In general it is felt that the shifts in potential do in fact represent the resting potential of the individual cells for the following reasons

1 The potential appears abruptly when the electrode is advanced a very small distance remains constant presumably while the electrode is advanced farther within the cell and then disappears abruptly during a further small displacement, presumably as the electrode leaves the cell

2 If one electrode is inserted in the cell and another one is inserted in the same cell as close as possible to the first, no change in the potential recorded by the first electrode is seen (Draper and Weidmann 1951) this observation shows that the insertion of an electrode does not in itself alter the resting potential to a measurable extent

3 The transmembrane potential recorded by an intracellular electrode is altered by all the various agents which are known to alter the demarcation potential (Hodgkin, 1951)

4 The transmembrane resting potential recorded with an intracellular electrode exceeds the demarcation potential by a considerable amount This would be expected from the shunting of the demarcation current through the extracellular fluid

5 When the transmembrane potential recorded with an intracellular electrode is excessively low, it is often noted that the electrode is broken and that its tip is large enough to have damaged the cell membrane

6 All of the observations on resting potential made with small electrodes which puncture the cell membrane are comparable to those made on the giant axon of the squid with an electrode inserted directly into the axoplasm through the cut end of the fiber (Hodgkin, 1951)

7 The number of cells judged from the number of resting potentials recorded during the penetration of several layers of muscle accords reasonably well with the number of cells determined by histological examination (Creese et al 1958)

The resting potentials recorded from the heart muscle of various species and from different functional types of cardiac cells range from 50 to 95 mv (Cranefield and Hoffman 1958*a*) The exact quantitative meaning of the values for the resting potential obtained in this manner has been subject to study and criticism (Adrian, 1956, Shanes, 1958) it is generally agreed that if the electrode is sufficiently small and is filled with 3 molar KCl the observed value of the potential may be in error by not more than a few millivolts

The Action Potential If a microelectrode is inserted into a cardiac cell and the cell is excited, the transmembrane action potential is recorded The shape and amplitude of the action potential vary considerably from one species to another and from fiber type to fiber type In general the action potentials recorded from all cardiac fibers resemble those of other excitable cells in showing an initial rapid depolarization or upstroke They differ however from the action potentials of most other excitable cells in showing a prolonged depolarization and a slow delayed repolarization Schematic action potentials of three types are shown in Fig 1 1 The action potential in Fig 1-1*A* is typical of many types of ventricular cells The initial rapid upstroke is labeled phase 0 a phase of early rapid repolarization is labeled phase 1 a prolonged phase of slow repolarization (often called the plateau) is labeled phase 2 the terminal phase of rapid repolarization is labeled phase 3 and the diastolic period is labeled phase 4 An action potential from a spontaneously rhythmic pacemaker fiber is shown in Fig 1 1*B* This action potential is characterized by the presence of a slow depolarization during phase 4 which eventually reaches threshold, this results in excitation and in the initiation of the upstroke of the locally arising action potential In such fibers the upstroke velocity seen in phase 0 is usually somewhat low The action potential shown

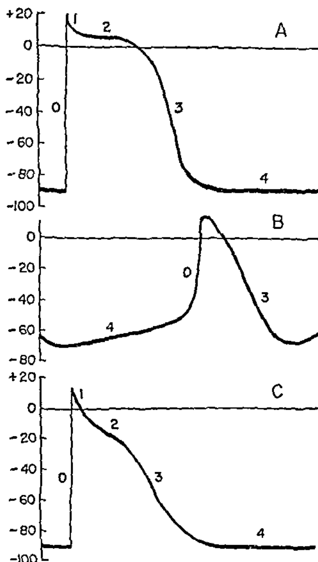


FIG 1 1 Schematic records of transmembrane action potentials recorded from ventricle (*A*) sinoatrial node (*B*) and atrium (*C*) Sweep velocity in *B* one half that in *A* and *C* Ordinate scale in mv See text for discussion

in Fig 1 1C is of the form recorded from most mammalian atrial fibers and from ventricular fibers of certain small mammals which do not show an isoelectric ST segment in their electrocardiogram In such fibers there is little or no plateau Phase 2 of repolarization is slower than phase 3, but it may be quite brief and its slope is sufficiently steep so that it can hardly be called a plateau The period of initial rapid repolarization (phase 1) is sometimes present and sometimes absent it is particularly prominent in fibers from the sinoatrial ring bundle the His bundle, and Purkinje system, and the avian ventricle

Attention must be called to the fact that during the terminal phase of rapid depolarization as well as during the initial part of the period of repolarization, the inside of the cell becomes positive with respect to the outside The degree to which this occurs, i e, the number of millivolts by which the inside becomes positive with respect to the original reference potential is known as the overshoot or reversal An overshoot of 15 to 20 mv is normally recorded from fibers of atrium and ventricle In the case of the spontaneously rhythmic pacemaker on the other hand the overshoot is normally reduced or absent The overshoot is of great importance for modern theories of the nature of the action potential and is discussed in detail in Chap 2

The Transmembrane Action Potentials of Specific Fiber Types

Each fiber type discussed below is the subject of an entire chapter in this book The survey given here is intended merely to provide a general orientation as a background for the treatment of the ionic theory given in the next chapter

The Atrium Action potentials which are typical of two of the fiber types found in rabbit atrium (Paes de Carvalho et al 1959) are shown in Fig 1 2 In Fig 1 2B the action potential of the contractile fibers which make up the atrium proper is shown It can be seen that there is no diastolic depolarization that the upstroke is rapid and that there is no well marked phase of initial rapid repolarization (phase 1) There are slow and rapid phases of repolarization corresponding to phases 2 and 3 but the phase of slow repolarization is too rapid to be called a plateau Figure 1 2A and C shows the type of action potential which can be recorded from specialized atrial fibers These fibers occasionally show pacemaker

activity and slow depolarization during phase 4 In the absence of slow diastolic depolarization the upstroke ends in a peaked overshoot, and there is commonly a relatively well marked phase 1 as well as a clearly recognized plateau

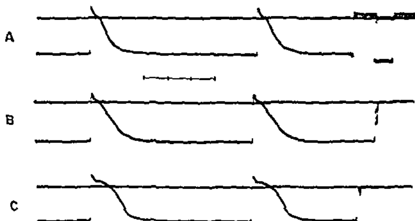


FIG 1 2 Transmembrane action potentials recorded from single atrial fibers of rabbit heart Top trace in each record is line of zero potential Vertical signal at right in *A* represents a voltage calibration of 100 mv (*A*) Fiber of cri ta terminalis (*B*) fiber of musculi pectinati (*C*) fiber of sinoatrial ring bundle Electrode withdrawn at end of records in *B* and *C* to check zero reference level Time calibration between *A* and *B* represents intervals of 100 msec

The Ventricle It is extremely unusual for ventricular fibers to develop pacemaker activity or to show any diastolic depolarization The action potential of dog papillary muscle seen in Fig 1 3*B* shows no diastolic depolarization, a rapid upstroke, a slight phase of initial rapid repolarization and a rather well marked plateau Such action potentials are characteristic of ventricular muscle from the majority of mammals In rats however, it is common to see potentials similar to those shown in Fig 1 3*A*, in which the phase of 'slow repolarization' is rapid so that there is no clear plateau This potential, recorded from the papillary muscle of a rat, resembles in certain respects those recorded from mammalian atrium

Fibers of the Conducting System The Purkinje fibers of the mammalian heart show an action potential distinctly different from that of ventricular muscle The action potential is prolonged, and this increase in duration results chiefly from the presence of a

well marked plateau or phase 2 (Draper and Weidmann, 1951) The phase of initial rapid repolarization (phase 1) is also well marked, so that the membrane potential during phase 2 is often near the reversal point Purkinje fibers also have a tendency to develop slow diastolic depolarization and consequent pacemaker

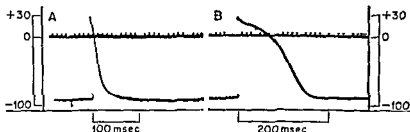


FIG 1-3 Transmembrane action potentials recorded with an intracellular microelectrode from a single ventricular fiber of rat heart (*A*) and dog heart (*B*) Upper trace in each record is line of zero potential and shows time marks at intervals of 10 and 50 msec Ordinate shows voltage calibration in millivolts



FIG 1-4 Transmembrane potentials recorded from two sites in an isolated preparation of dog Purkinje fibers (*A*) Control note slow depolarization during phase 4 terminating in a steady level of resting potential (*B*) and (*C*) changes induced after addition of epinephrine note increase in slow depolarization during phase 4 appearance of pacemaker activity at site of recording electrode shown on lower trace in *C* and the associated changes in the rising phase and amplitude of the action potential

activity Figure 1-4*A* shows an action potential of a normal Purkinje fiber of a dog heart, Fig 1-4*B* and *C* show an action potential from the same fiber after addition of epinephrine and development of marked depolarization during phase 4

Fibers of the Sinoatrial Node As might be expected, fibers of the sinoatrial node show well developed spontaneous depolarization during phase 4 This spontaneous depolarization is the basis for the pacemaker activity shown by this tissue (West, 1955*a* *b*) Action

potentials from these fibers are distinctive in many other ways (Fig 1 5) The rising velocity of the upstroke is usually low, the resting potential is reduced the overshoot is diminished or absent, and the phases of repolarization differ from those seen in atrium ventricle, or Purkinje fibers A distinction is made between fibers which show spontaneous diastolic depolarization and fibers which

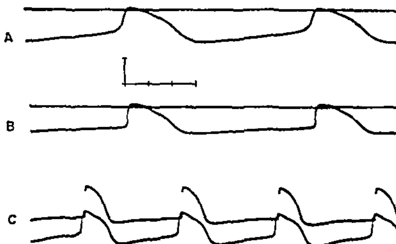


FIG 1 5 Transmembrane potentials recorded from single fibers of the sinoatrial node of rabbit heart (*A*) True pacemaker fiber showing gradual transition from phase 4 to phase 0 Top trace is line of zero potential (*B*) Latent pace maker showing more abrupt transition from pha es 4 to 0 (*C*) Simultaneous records at a slower sweep velocity from pacemaker fiber (bottom trace) and latent pacemaker (top trace) Calibration between *A* and *B* represents 50 mv and 100 msec See text for discussion

act as true pacemakers This distinction is based upon the transi tion from phase 4 to phase 0 If the spontaneous diastolic depolariza tion proceeds all the way to threshold so that phase 4 and phase 0 merge smoothly, the fiber is thought to have reached threshold as the result of its own intrinsic depolarization and is said to be a true pacemaker (Fig 1 5*A*) On the other hand if the slow diastolic depolarization is abruptly interrupted by the appearance of a rapid upstroke (phase 0) as is seen in Fig 1 5*B*, the fiber is believed to have been excited by a propagated impulse before its own depolari zation reached threshold Such fibers are said to show pacemaker activity and are regarded as latent pacemakers

THE CARDIAC ELECTROGRAM

Records obtained from cardiac muscle by means of unipolar or bipolar leads in close proximity to active uninjured tissue are called *cardiac electrograms* to distinguish them from electrocardiograms, which are recorded with leads distant from the heart The electrogram indicates the potential difference between two points on the surface of the tissue or between one point on the tissue and a remote point The potential difference results from activity of, or injury to the tissue under study An electrode close to the tissue is often referred to as the *active lead* a remote electrode is usually called an *indifferent lead* this term does not mean, however, that the remote lead is not influenced by changes in potential originating in the tissue

The unipolar electrogram is recorded with one lead close to or in contact with cardiac muscle and the other lead at some remote point The bipolar electrogram employs two o called active leads both in close proximity to the tissue in some cases these leads may be quite widely separated on the muscle, but in most instances they are kept as close together as possible In this latter case they have been described as contiguous bipolar electrodes (Harris 1941) or differential electrodes In general, the unipolar electrode is used to obtain information concerning the voltage time course of the ST segment and T wave contiguous bipolar electrodes are most frequently employed to time the arrival of activation at one site However there are a number of points which should be considered in modification of these general principles

The Unipolar Electrogram

Typical unipolar electrograms recorded from a strip of parallel cardiac muscle fibers immersed in a relatively large volume of electrolyte are shown in Figs 1 6 and 1 7 As can be seen the record shows a pair of biphasic complexes the R and T waves separated by an isoelectric ST segment The polarity employed in these records is similar to that used in clinical electrocardiography with positivity of the 'active' electrode recorded above the line of zero potential In Fig 1 6A the record of the transmembrane potential of a single fiber has been simultaneously recorded from a location immediately adjacent to the unipolar surface electrode It can be

seen that the initial complex of the electrogram corresponds in time to the upstroke of the transmembrane action potential and the T wave to the phase of repolarization Close inspection of the same activity recorded at a higher sweep velocity shows that the rapid

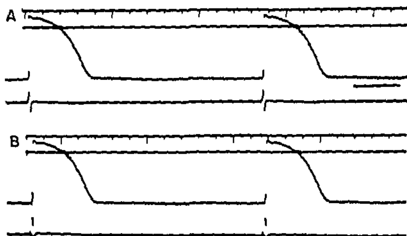


FIG 1 6 Transmembrane action potentials recorded from dog papillary muscle simultaneously with unipolar (*A*) and bipolar (*B*) electrograms Top trace in *A* and *B* shows time marks at intervals of 100 and 500 msec Second trace from top in *A* and *B* shows line of zero potential Driving stimulus artifact precedes el ctrogram deflections Horizontal bar at right of *A* shows voltage calibration of minus 100 mv See text for discussion

plus-to minus deflection of the initial complex is virtually simul taneous with depolarization of the underlying fiber shown in the upstroke of the transmembrane action potential This record demonstrates that as would be expected (Macleod 1938) the intrinsic deflection of the electrogram signals the time of depolariza tion of tissues in the immediate vicinity of the active electrode When on the other hand the distant electrode is close to the heart or otherwise poorly located the meaning of the intrinsic deflection is less certain Similarly if the active electrode makes firm enough contact with the heart muscle to cau e even slight local injury the rapid po itive-negative deflection may no longer indicate the onset of local depolarization These two difficulties have led recent investi gators to minimize the value of the unipolar electrogram in timing local excitation The effect of distance of the tissue from the active electrode is also worth mentioning As the active electrode is moved

farther and farther from the tissue the magnitude of the potential difference resulting from activity decreases rapidly More important the rapid positive-negative deflection becomes less and less accurate as an indicator of the instant at which underlying tissue is depolarized (Medrano et al, 1957, Itatsu 1954)

The unipolar electrogram is quite useful for studying the direction and in some instances also the velocity of the spread of activity in cardiac muscle The three records shown in Fig 1 7 have been obtained at one end in the middle and at the other end of a strip of parallel fibers When the recording electrode is at the site of origin of activity the initial complex of the electrogram shows only an initial rapid negative deflection followed by a slower return of the trace to the zero line The record from the middle of the strip shows a slow, positive deflection as activity approaches the electrode, a rapid positive-negative deflection synchronous with local depolarization and a slow return of the trace to the zero line as activity recedes from the electrode Finally when the electrode is located on the last part of the strip to be activated the electrogram shows only a slow positive deflection which terminates in a sudden return of the trace to the zero line as activity reaches the electrode site Thus whether used to study a small isolated preparation of heart muscle or local activity in the intact organ (Siebens et al 1951) the unipolar electrogram usually gives useful information on the position of the

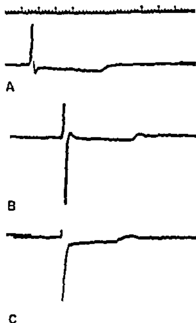


FIG 1 7 Unipolar electrograms recorded from an isolated dog papillary muscle immersed in a large volume of Tyrode solution Top trace in *A* shows time calibration at intervals of 10 and 50 msec *A* *B* and *C* show the unipolar electrogram recorded from one end from the middle and from the other end of the muscle Driving stimuli applied to muscle at end employed for *C* See text for discussion

electrode relative to the point of origin of activity and the direction of the spread of depolarization relative to the electrode Changes in the velocity of propagation can be estimated from changes in the total duration of the R wave, but for accurate measurements of this sort two or more simultaneous records (unipolar or bipolar) from different locations are more satisfactory

The unipolar electrogram gives the most useful records of the *ST* segment and T wave As can be seen in Fig 1-7, when records are obtained from a muscle strip the T wave is opposite in polarity to the initial deflections The magnitude of the T wave varies directly with the rate of repolarization and with the temporal dispersal of repolarization throughout the preparation This is illustrated in Chaps 3 and 4 The duration of the T wave corresponds in a less accurate manner to the duration of the state of nonhomogeneous polarization during recovery The unipolar electrogram gives a useful indication of the voltage time course of the transmembrane potential during the *ST* segment In most records this segment is displaced somewhat from the line of zero potential and the amount of displacement varies with the rate of change of membrane potential during the plateau similarly local differences in the slope of the plateau are reflected in appropriate changes in the position and the shape of the *ST* segment

The Bipolar Electrogram

The bipolar electrogram (Fig 1 6*B*) recorded with contiguous differential electrodes is used primarily to time activation of a localized area of muscle When the mass of cardiac tissue is large this technique is often superior to the unipolar record On the other hand when the propagation velocity is low as in the sinoatrial and atrioventricular nodes contiguous bipolar electrodes are of little use since the potential difference between the two electrodes is negligible For the same reason the T wave obtained with close bipolar electrodes is useful only for rough timing of repolarization Furthermore the shapes of the R and T complexes are more difficult to interpret than those obtained with unipolar recording techniques The amplitude of the R and T waves of the electrogram recorded with close bipolar electrodes depends primarily on the direction of spread of activity when this spread is at a right angle to the line joining the electrodes the amplitude of the deflection is minimal

In some instances widely separated bipolar leads can be employed to record activity at two different sites on one channel of the recording device Finally, if the bipolar electrode is in contact with injured tissue the records obtained cannot be employed to time local activation Under a condition where injury due to pressure of the electrode on the tissue is likely the unipolar technique is always preferable since in the bipolar electrogram the effects of injury which normally appear in the *ST* segment and *T* wave may be obscured Furthermore, local injury will result in slowing of the intrinsic deflection of the unipolar record, while with bipolar electrodes the resulting change in amplitude of the R wave may be interpreted as resulting from a change in the direction of spread of activity In summary electrograms recorded by means of close bipolar electrodes find their greatest usefulness in timing local activity in either the intact heart or in large masses of cardiac muscle

Before concluding the section on the cardiac electrogram it should be noted that records obtained with capacitor coupled amplifiers are generally unsatisfactory Not only are the slow voltage changes of the T wave and *ST* segment obscured but also slowly spreading depolarization similar to that of the sinoatrial node region is less readily recorded

The Relationship between the Transmembrane Potential and the Electrogram

Many efforts have been made to relate the shape of the transmembrane potential to the shape of the electrogram Little however has been added to the general idea advanced by Burdon Sanderson and Page (1880 1884) that the electrogram is in some mathematical sense a derivative of the monophasic potential (Fig 1 6) It seems necessary to remark that efforts to reconstruct the shape of the transmembrane potential from the shape of the electrogram have become pointless now that the transmembrane potential can be measured directly It is furthermore noteworthy that such a reconstruction requires a knowledge of the membrane characteristics, conduction velocity and syncytial structure which is on the whole lacking

There are nevertheless certain rules of thumb which are useful in understanding in a qualitative manner why certain changes in the

transmembrane action potential result in certain changes in the electrogram It is roughly true that the more rapidly the voltage change across the membrane occurs, the greater the amplitude of the corresponding complex in the electrogram will be This is true for electrograms obtained with either unipolar or close bipolar leads This rough rule explains the difference in amplitude of the R wave and the T wave in the electrogram The amplitude of the T wave would be expected to approach the amplitude of the R wave if repolarization were to occur as rapidly as does depolarization A change in this direction can in fact be noted when atrial repolarization is accelerated by acetylcholine (Fig 3 5) Generally speaking the rapidity of conduction influences the amplitude of the electrogram recorded with close bipolar leads In principle if the steepness of the change of transmembrane potential at a point remains constant and conduction velocity is slowed the amplitude of the corresponding component in the electrogram will be increased

The Monophasic Action Potential

It is possible to record a monophasic action potential with surface electrodes if one electrode is in intimate contact with injured tissue Although injury can be produced by a number of means (cutting crushing burning pressure application of concentrated KCl, etc) in most instances signs of injury rapidly disappear However, if injury is produced by sucking a bit of the cardiac muscle into the recording electrode and if the suction is maintained (Eyster, Meek, Goldberg and Gilson 1938) monophasic action potentials of constant amplitude and shape may be recorded for many minutes Records obtained by this technique do not show the true transmembrane potential They do, however, record the demarcation, or injury potential which is a variable fraction of the resting potential These records also give a reasonably accurate indication of the voltage time course during activity with certain exceptions noted below When the record obtained by the suction electrode is compared to the record of transmembrane potential recorded with an intracellular microelectrode (Figs 1 8, 1 9), the following points are clearly seen First the magnitude of the suction electrode record is smaller than the transmembrane action potential and the relative magnitude of the reversal or overshoot is different (Fig 1 8) Second, the upstroke of the suction electrode record is slower and

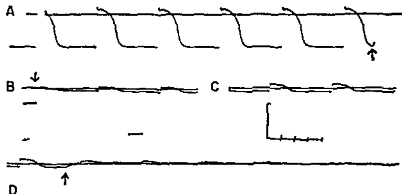


Fig 1 8 Comparison of the overshoot or reversal of potential as recorded with a microelectrode and a suction electrode from an isolated cat papillary muscle In *A* a microelectrode record is shown with a zero reference line At the end of the record (arrow) the electrode is withdrawn to check the reference potential In *B* and *C* the slow development of a monophasic potential is shown during several beats after the application of suction (arrow) to a small suction electrode In *D* the suction is released (arrow) to check the reference potential A small monophasic action potential persists for a few beats after the release of suction Calibration represents 100 mv and 200 msec (*Hoffman Cranefield Lepeschkin et al* 1959)

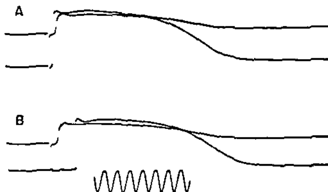


Fig 1 9 Comparison of transmembrane action potentials recorded through an intracellular microelectrode (dotted upstrokes) and monophasic potentials recorded *simultaneously with a suction electrode from* cat papillary muscle Similar amplification employed for both types of records Sine wave calibration 60 cps 50 mv in amplitude (*A*) Both electrodes located close to the origin of activity (*B*) microelectrode moved to opposite end of muscle See text for discussion (*Hoffman Cranefield Lepeschkin et al* 1959)

sometimes notched (Figs 1-9, 1-10) Finally, the remainder of the monophasic action potential accurately depicts the rate of change of membrane potential during recovery (Fig 1 10)

The suction electrode may be paired with another electrode which is remote from the heart to obtain a unipolar monophasic record



FIG 1 10 Comparison of monophasic action potentials recorded very close together through an intracellular microelectrode (top figure) and a suction electrode (middle figure) The gains are adjusted to make the apparent amplitudes the same In the bottom figure the two traces are superimposed to show the similar time course of repolarization A stimulus artifact is apparent in all records before the upstroke of the action potential (*Hoffman Cranefield Lepeschkin et al* 1959)

It may also be paired with an electrode on the heart muscle which is very close to the injured area to obtain a bipolar or differential record In either case a monophasic action potential will be obtained the rapid upstroke of which corresponds to the time of arrival of activity at the site of the suction electrode (Fig 1 9) (Hoffman, Cranefield Lepeschkin, et al, 1959) When the suction electrode is paired with another electrode distant from the site of injury, the monophasic action potential is distorted to a greater or lesser extent by the electrical activity recorded by the distant electrode Although the differential suction record can be employed to time the arrival of excitation at the electrode site it is not the technique of choice However the suction electrode is very convenient for

determining the time course of repolarization It is particularly well suited to the study of tissue in which it is difficult to use intra cellular microelectrodes, such as the intact hearts of large animals and most hearts in situ where movement of the heart breaks or dislodges the electrode tip

EXCITATION OF HEART MUSCLE

It is often desirable to study the effects of current flow on heart muscle In addition to the use of suprathreshold electrical driving stimuli to initiate activity in quiescent preparations pulses of cur rent are used to determine the threshold of heart muscle at different times throughout the cycle of activity (Brooks et al 1955) In a more general sense the effect of the passage of current through the membrane of an excitable cell either at rest or during the action potential may provide important information about the nature of the membrane properties which underlie excitability Thus the passive electrical properties of cardiac cells are measured by the use of very weak electrical polarization of the fibers (Weidmann 1952) the local response is revealed by use of subthreshold electrical stimuli (Kao and Hoffman 1958) and the nature of repolarization has been partially clarified by the use of anodal current pulses applied to the fiber during the action potential (Cranefield and Hoffman 1958*b*)

The use of electrical stimuli to study cardiac excitability has recently been described in detail (Brooks et al 1955 Cranefield Hoffman, and Siebens 1957) If one or more intracellular micro electrodes are employed during stimulation experiments it is possi ble to measure the change in membrane potential which results from the stimulus current moreover if one of the intracellular microelectrodes is used as a stimulating electrode one can not only study the excitability of a single cell but in addition obtain informa tion about the passive and active properties of the membrane with a degree of accuracy beyond the reach of other methods In subse quent chapters specific aspects of stimulation technique will be described in relation to their application to certain problems in *cardiac electrophysiology*

2

EXCITATION AND CONDUCTION

Excitation and conduction the two aspects of cardiac electro physiology with which this book is chiefly concerned, depend upon somewhat different properties of the cell In a certain sense, excitability is the more fundamental property, it may exist in cells which do not conduct It is a property of specialized membranes, and it appears to rest upon the ability of such membranes to maintain certain specific permeabilities to ions and to alter those permeabilities in a special manner Cellular metabolism enters into excitability in two quite different ways It provides energy sources for the maintenance of the structural and functional properties of the membrane It also provides energy for the maintenance of an unequal distribution of ions between the interior and exterior of the cell In principle the fundamental properties of an excitable membrane are independent of this latter aspect of cellular metabolism and could be revealed merely by placing the membrane between two solutions of the appropriate composition Because of that fact and because little is known about the role of metabolism in sustaining the membrane or in sustaining the ionic gradients attention is directed to a consideration of the permeability changes as the basis for membrane excitability

Conduction depends upon the fact that certain cells which have an excitable membrane also have anatomical and electrical properties which ordinarily ensure that if one area of the membrane is excited to full activity that area will excite adjacent areas The electrical properties of the extracellular fluid the cytoplasm and the membrane are all involved in providing a mechanism which permits conduction

ANATOMICAL AND PHYSICAL ASPECTS OF CONDUCTION

The Anatomy of Excitable Cells

The ultrastructure of excitable cells has recently been studied rather intensively by means of several improved techniques, and the resulting excellent descriptions of the fine structure of nerve (Fernandez Moran and Brown 1958) and skeletal muscle (Porter 1956, Bennet, 1956, Porter and Palade 1957) have permitted certain tentative correlations between structure and electrical activity (Hanson and Huxley 1955 Hodgkin 1957 Huxley 1957 Huxley and Niedergerke 1959, Huxley and Taylor 1958) Electron-microscopic observations on fibers of the cardiac syncytium (Moore and Ruska, 1957, Muir 1957*a* and *b*) similarly provide a reasonably clear demonstration of the fine structural details of cardiac muscle

The outer limiting membrane of cardiac fibers the sarcolemma, appears to consist of two dense layers separated by a less dense structure or space This complex is approximately 200 to 300Å in thickness The outer layer of the sarcolemma the basement membrane appears to be uninterrupted for considerable distances The inner layer or plasma membrane in contrast seems to be continuous with certain intracellular structures such as the intercalated disks and endoplasmic reticulum The former of these structures is composed of two layers of plasma membrane separated by a space similar in appearance and width to the space between the inner and outer layers of the sarcolemma The endoplasmic reticulum is a complex tubular structure which is in intimate contact with the nuclei and myofibrils and with the sarcolemma at the level of the Z and M bands The lumen of the endoplasmic reticulum is thought to be continuous with the clear space which separates the inner and outer dense layers of the sarcolemma Other aspects of the anatomy are quite similar to skeletal muscle All the classical bands of skeletal muscle are present in cardiac fibers the myofibrils are separated by dense chains of mitochondria and are seen in cross section to be composed of regularly spaced filaments Two aspects of the structural details revealed in these studies are of particular importance in relation to the electrical properties of cardiac muscle Anatomical evidence strongly indicates that the intercalated disks are not crossed by the myofibrils or other intracellular elements and thus

effectively divide the cardiac syncytium into separate cells or units If the intercalated disks have an electrical resistance comparable to that of the membrane of other excitable cells, the disks would be expected to have an appreciable effect on the longitudinal flow of current and thus on conduction Also, if the inner layer of the sarcolemma is the site of the selective permeability which characterizes excitable membranes it might be expected that the currents associated with electrical activity would have a distribution corresponding to the anatomy of the endoplasmic reticulum Finally, the relatively high electrical capacity of the cardiac membrane as calculated from the external dimensions of the fiber might result from the great area of membrane afforded by the intercalated disks and endoplasmic reticulum (Moore and Ruska 1957)

At present experimental data, both anatomical and electrophysiological, do not permit an answer to these questions In this chapter the term *membrane* will be employed to designate a structure or group of structures which possess certain electrophysiological characteristics and the cardiac syncytium will be considered as composed of long uninterrupted fibers i e it will be assumed that the intercalated disks do not represent a major barrier to the flow of longitudinal current It is likely that new information will require some modification of both these usages

Passive Electrical Properties of Excitable Cells

The Extracellular Fluid and the Cytoplasm Both the extracellular fluid and the cytoplasm conduct electric current This is so because of the presence of small inorganic ions and also to some extent because of the presence of larger organic ions The resistance of the extracellular fluid may be varied artificially (e g , by replacement of the extracellular fluid by isotonic sucrose) but in general its resistance is less than that of the intracellular fluid Potassium ions make up a large part of the small mobile charged particles in the cytoplasm The general opinion is that some intracellular K^+ is bound to protein but that most of it is diffusible much of the flow of current in the cytoplasm thus depends on movement of this ion

Membrane Resistance The fact that a potential difference of about 100 mv exists across the membrane of excitable cells shows that the cellular membrane possesses a resistance to current flow

Since the flow of current in living tissues depends upon the movement of charged ions the membrane resistance represents the permeability of the cell to charged ions Membrane resistance can be determined by passing current through cells either with the aid of extracellular electrodes or intracellular electrodes If one microelectrode is placed inside a cell and another is placed just outside the application of an emf between the two electrodes will result in less flow of current than if both electrodes are either inside or outside the cell This observation shows that the resistance of the membrane is higher than that of the extracellular or intracellular fluid Measurements of this sort have been made for heart muscle and exact values for membrane resistance where known, are given in the chapters on the various fiber types

Membrane Capacity If a small current is suddenly applied across the membrane of an excitable cell it is found that the transmembrane potential does not change abruptly but reaches a new value rather slowly At the polarizing electrode the transmembrane potential reaches its new value with an exponentially delayed rise (see Fig 2-1) It is therefore apparent that the excitable membrane pos esses capacity The exact values of membrane capacity may be of great importance in some fibers since a sufficiently high capacity may slow conduction velocity Also the presence of both resistance and capacity in the membrane confers on the fiber its so-called core-conductor properties

The Core Conductor The excitable cell is more or less cylindrical and is very long in relation to its diameter The cytoplasm conducts current as does the extracellular fluid which surrounds the cell The presence of a resistive and capacitative membrane means that the cell possesses the same electrical properties as a telegraph cable or core conductor and may be represented by the diagram shown in Fig 2 2 The cable or core-conductor properties of the excitable cell are of considerable importance in conduction The fact that the transverse resistance of the membrane is higher than the longitudinal resistance of the extracellular fluid or the cytoplasm is especially important since it means that the flow of current which results from the application of an emf at one point spreads along a relatively great length of fiber This spread of current is referred to as *electrotonic spread*

The two cable properties of chief interest are the time constant

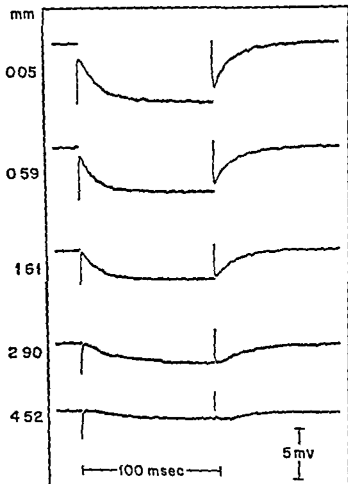


Fig 2-1 The electronic spread of a 100-msec anodal pulse applied to a single Purkinje fiber The transmembrane potential is recorded at 0 05 0 59 1 61 2 90 and 4 52 mm from the applied polarization (*Weidmann* 1952)

and the length constant Both may be evaluated from the type of information shown in Fig 2-1 The length, or space constant determines the degree to which the steady state value of the electrotonic potential falls off with increasing distance from the applied voltage In a cable this fall-off is expressed by the simple relationship

$$P = P_0 e^{-x/\lambda} \qquad (2\ 1)$$

where x = distance from applied potential
P = displacement of membrane potential at x
P_0 = displacement of membrane potential at site of voltage application
e = base of natural logarithms
λ = length constant

Since the final steady state values of P are considered membrane capacity does not affect the value of λ From Eq (2 1) it follows that the electrotonic potential falls off by $1/e$ in one space constant

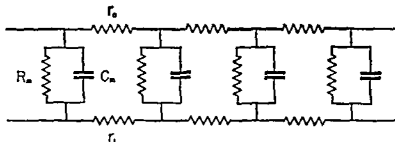


FIG 2-2 A circuit model of the excitable cell R_m membrane resistance C_m membrane capacity r_o extracellular fluid resistance r_i cytoplasmic resistance See text for discussion

The space constant for Purkinje fibers is about 2 mm (Weidmann 1952) so that the electrotonic displacement of membrane potential 6 mm from the site of applied voltage would be $1/e^3$ or about 5 per cent of the applied change

The membrane time constant τ_m is defined by the relationship

$$\tau_m = R_m C_m \tag{2-2}$$

where C_m = membrane capacity, $\mu f/cm^2$
R_m = membrane resistance, ohm-cm²

The significance of τ_m is that a small displacement of potential leaks away to $1/e$ of its initial value in one τ_m

Conduction Conduction of excitation depends upon the core-conductor properties of the excitable fiber and upon the ability of the membrane to develop a rapid change of potential When a small area of membrane is depolarized by the application of a cathodal stimulus the depolarization rapidly becomes regenerative so that the excited point reaches a very different transmembrane potential

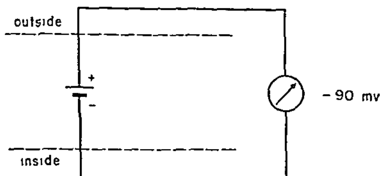
from that of the unexcited fiber The excited point in fact becomes a cathode with respect to the unexcited fiber The flow of current between excited and unexcited tissue is directed along the length of the fiber by virtue of the core conductor properties so that the region adjacent to the excited site is depolarized sufficiently to become excited itself Conduction of excitation along the entire fiber thus results It will be seen that conduction may be impaired either by a decrease in the amplitude of the action potential, i e by reduction of the degree of depolarization at the excited point or by a change in the core conductor properties such that longitudinal spread of the depolarizing current is impaired Thus conduction velocity can be reduced by increasing the resistance of the extracellular fluid, if the resistance of either extracellular fluid or intracellular fluid were to become great enough, conduction would fail entirely

THE IONIC BASIS OF THE RESTING AND ACTION POTENTIALS

We have seen that the primary event in conduction of an impulse down an excitable fiber is the development of depolarization at the excited point The depolarization so developed serves to excite the adjoining area of the membrane and so on along the length of the fiber In the intact heart certain areas show spontaneous depolarization, so that waves of depolarization regularly pass over the heart in the absence of any external stimulus As was shown in Chap 1, the excited area of the cell is not merely depolarized but in fact a reversal of polarization is seen This reversal which was first detected in the giant axon of the squid has now been seen in nearly all excitable fibers The theory developed to explain this reversal in the giant axon (Hodgkin 1951, Hodgkin 1957) has had so much influence on recent experimental studies of the electrophysiology of the heart that it is necessary to examine it at this point The presentation which follows is a simplified version of the theory the vast majority of evidence for which has been derived from studies of fibers other than those of the heart It should be emphasized that this theory has not by any means been shown to be generally applicable to the heart Nevertheless in recent years it has occupied a dominant position in influencing the planning and interpretation of experiments concerned with cardiac electrophysiology

An Electrical Model of the Membrane

If we examine an excitable fiber with the aid of intracellular recording we find that, when the fiber is at rest, its interior is about 90 mv negative with respect to the exterior of the cell The potential difference across the resting membrane may therefore be represented by the simple diagram seen in Fig 2-3A If on the other hand, the fiber is conducting an impulse as the impulse



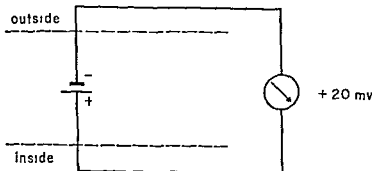
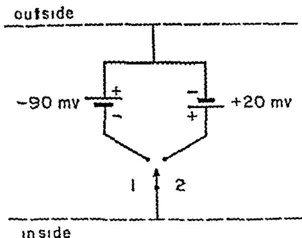


Fig 2-3 Elementary model of the membrane (A) The resting fiber (B) the active fiber See text for description



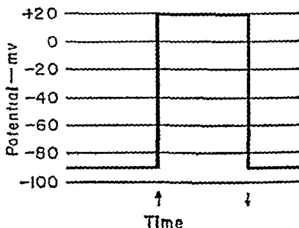


FIG 2-4 (*A*) Elementary model of the membrane Key in position 1 corresponds to the resting fiber key in position 2 corre ponds to the active fiber (*B*) The action potential generated by the model shown in *A* as the key is switched from position 1 to 2 and then back to position 1 at times indicated by arrows

passes the microelectrode, it is found that the inside of the fiber is about 20 mv positive with respect to the outside Thus the state of the membrane at the point of maximal activity may be represented by the diagram shown in Fig 2 3*B* It is apparent that two batteries of proper strength which are connected, as shown in Fig 2-4*A*, with a key which would throw first one battery and then the other into the circuit could serve as a simple model of these two

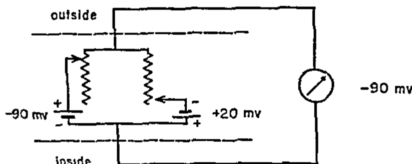


Fig 2 5 Model of the membrane corresponding to a resting fiber See text for discussion

tates of the membrane The "action potential" produced by such a model would consist (Fig 2-4*B*) of a resting potential of 90 mv (inside negative) seen when the key is in position 1 an instantaneous reversal to a plateau of 20 mv (inside positive) when the key is in position 2 and an instantaneous "repolarization" to minus 90 mv when the key is returned to position 1

All that is needed to mimic faithfully the shape of an action potential besides two such batteries is a means of achieving a smooth transition from the stage at which one battery dominates the system to the stage at which the other one does so This can be achieved by using two resistances capable of large variation one in series with each battery as shown in Fig 2-5 This arrangement of electromotive forces and resistances is a somewhat unfamiliar one but it can readily be seen that by a suitable adjustment of the resistances any level of potential between the extremes may be obtained Furthermore, by imposing a suitable change in resistances as a function of time, a suitable change in potential as a function of time will result

The Ionic Basis of the Membrane EMF

The question arises, therefore, whether or not anything can be found in the excitable cell which might correspond to the circuit shown in Fig. 2.5. A possible source of potential difference is readily found in the uneven distribution of inorganic ions and in particular in the distribution of potassium and sodium. Under suitable conditions any uneven distribution of inorganic ions in an aqueous solution can act as a battery or concentration cell (see Hober, 1945, Davson 1951, Bayliss 1924). If, for example, solutions containing unequal concentrations of K^+ are separated by a membrane selectively permeable to K^+, it will be found that the potential which arises can be described by the Nernst relation

$$E_K = \frac{RT}{F} \ln \frac{[K_i^+]}{[K_o^+]}$$

or at 37°C

$$E_K = 61.5 \log \frac{[K_i^+]}{[K_o^+]} \qquad (2.3)$$

in which E_K is the potential difference attributable to the concentration difference of K^+. K_i^+ and K_o^+ are the concentrations (strictly the activities) of K^+ inside and outside the fiber, and R, T, and F are the gas constant, absolute temperature, and the Faraday, respectively. Since it is possible in some excitable tissues to obtain rather accurate measurements of K_i^+ and K_o^+, we can predict quantitative values for E_K from the above formula. The values for potassium concentrations of cat heart muscle (Robertson and Dunihue 1954) are

$$[K_i^+] = 151 \text{ meq/l}$$
$$[K_o^+] = 4.8 \text{ meq/l}$$
$$[K_i^+]/[K_o^+] = 31$$

so that

$$E_K = 61.5 \log 31 = 92.6 \text{ mv}$$

On the other hand, the distribution of Na^+ is exactly opposite to that of K^+, in that there is much more Na^+ outside the cell than inside. In fact, the values for cat heart from the same study are

$$[Na_o^+] = 159 \text{ meq/l}$$
$$[Na_i^+] = 6.5 \text{ meq/l}$$
$$\frac{[Na_i^+]}{[Na_o^+]} = 1/24$$

so that $E_{Na} = 61.5 \log 0.042 = 84$ mv, inside positive

If therefore, we accept the sodium and potassium concentration gradients as a possible source of the two opposing electromotive forces which the excitable membrane apparently develops at rest and during activity, we may redraw Fig 2 2 by inserting E_K and E_N for the emf s (Fig 2 6) It will be noted that the value obtained from E_K is slightly larger than the value given for the transmembrane potential of the resting fiber and the value of E_N is much larger than the value of 20 mv given for the active fiber The reasons why this is so will become clear later

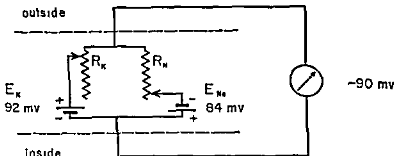


FIG 2-6 Model of the membrane corresponding to the resting fiber E_K the emf resulting from the K^+ concentration gradient R_K the resistance to flow of K^+ E_N the emf resulting from the Na^+ concentration gradient R_N the resistance to flow of Na^+

Having identified the sources of emf with the Na^+ and K^+ gradients we must ascertain the meaning of the variable resistances in the model If we consider a single element say the K^+ emf, and write it in terms of Ohm s law (Ohm 1827), we obtain

$$E_K = I_K R$$

where by I_K we indicate a current flow in which charge is carried by means of the movement of K^+ ions If the source of the emf is the unequal distribution of K^+ ions and if the current corresponding to this emf is carried by the movement of K^+ ions, then the resistance term must correspond to the resistance to the flow of K^+ ions through the membrane and we may write

$$E_K = I_K R_K$$

The resistance to flow of a specific substance through a membrane

is the reciprocal of the permeability, i e ,

$$R_K = \frac{1}{P_K}$$

where P_K is the K^+ permeability If the above equation is written in terms of conductance,

$$E_K = \frac{I_K}{P_K}$$

the conductance term P_K corresponds to the permeability of the membrane toward K^+ ions A similar analysis leads us to identify the other resistance term with the resistance to flow of Na^+ ions and to label our model as is done in Fig 2 6

In order to obtain a reasonably complete electrical model of a small unit of excitable membrane it is necessary to add an emf and a resistance term to allow for the contribution to the transmembrane potential of other ion species and to add a capacitative term to allow for the membrane capacity This model contains all the properties of the membrane necessary to describe the voltage changes associated with activity and recovery provided that the time course of the permeability changes is known If the core-conductor properties are included by adding the resistance of the cytoplasm and the resistance of the extracellular fluid, one obtains the model (Fig 2 7) on which the differential equations of the Hodgkin theory are based (Hodgkin and Huxley, 1952b, Hodgkin, 1957)

The aspect of this model with which we are primarily concerned at the moment is that part which corresponds to Fig 2 5 In Fig 2 7*A* we see the state of the model corresponding to the resting membrane The K^+ permeability is higher than the Na^+ permeability, and the transmembrane potential is dominated by E_K The transmembrane potential is less than E_K because the Na^+ permeability is not negligible so that there is an IR drop across the Na^+ circuit which opposes E_K Similarly in Fig 2 7*B* we see the state of the model corresponding to the active membrane at the point of maximum depolarization Here again the transmembrane potential is less than E_N because the K^+ permeability is still significant in magnitude compared with the Na^+ permeability

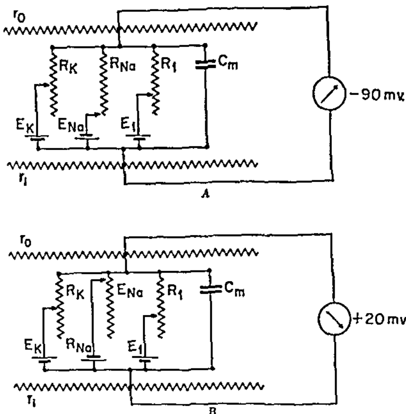


FIG 2-7 The model of the excitable cell on which the Hodgkin Huxley equations are based (*A*) The resting state of the fiber (*B*) the active state of the fiber E_K the emf resulting from the K^+ concentration gradient R_K the resistance to flow of K^+ E_N the emf resulting from the Na^+ concentration gradient R_N the resistance to flow of Na^+ E_1 the emf resulting from the concentration gradients of all other ions R_1 the resistance to flow of those ions r the resistance of the extracellular fluid r_i the resistance of the cytoplasm C_m the membrane capacity

The Potassium EMF and the Potassium Permeability The model constructed above says that the resting potential is made up primarily of the emf resulting from the K^+ gradient and that this is so because when the fiber is at rest the permeability to K^+ is enough higher than the permeability to other ions to permit domination of the transmembrane potential by the K^+ potential

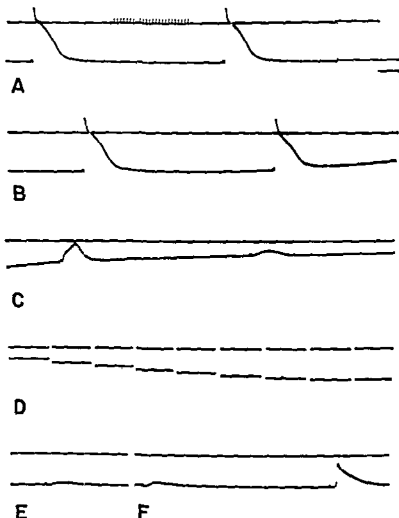


FIG 2-8 The effect of high external K^+ concentration on the resting and action potential of an atrial fiber Extracellular K^+ concentration is increased in *B* Maximum depolarization (and inexcitability) is seen in *C* Gradual repolarization as external K^+ is washed away is seen in *D* Return of excitability is seen in *E* and *F* Calibrations in *A* represent 100 cps and 100 mv See text for discussion

If this is true then it must follow that changes in the K^+ concentration gradient should change the resting potential In particular, increasing the concentration of K^+ in the extracellular solution should depolarize the fiber and it should be possible to predict the degree of depolarization by the Nernst equation Rather good agreement of this sort has in fact been obtained on many types of fibers (Hodgkin 1951) Figure 2 8 shows the effect on the transmembrane potential of a single atrial fiber of a transitory marked increase in the concentration of extracellular K^+ It will be noted that the depolarization leads to inexcitability, and the excitability returns gradually as the outside K^+ diffuses away and the resting potential rises Generally speaking the greater the K^+ permeability is relative to the permeability to other ions the nearer the transmembrane potential will approach that defined by the K^+ concentration gradient

The Sodium EMF and the Sodium Permeability The detailed sequence of permeability changes which underlies the conducted action potential will be discussed below but it is apparent from what has been said already that the crucial event associated with conduction is a sudden change in the relative values of the membrane permeability to Na^+ and K^+ so that during the upstroke of the action potential the Na^+ permeability of the fiber is much greater than the K^+ permeability Therefore the Na^+ emf dominates the transmembrane potential It follows that the amplitude of the action potential must depend on the Na^+ concentration gradient in exactly the same way that the amplitude of the resting potential depends on the concentration gradient of K^+ The Hodgkin theory also predicts that the rate of depolarization must depend on the external Na^+ concentration A diminution in both the amplitude and steepness of the rising phase can be seen in Fig 2 9 in which the effect of low external Na^+ on fibers of frog sartorius muscle is shown The degree to which this prediction is fulfilled in heart is discussed in the chapters on the fiber types as well as in Chap 9

The Sequence of Permeability Changes Underlying the Action Potential The amplitude of both the resting potential and the action potential can be changed by changing the concentrations of suitable ions, but during the action potential the voltage changes seen are *not* the result of changes in either E_K or E_N They are rather the result of changes in the relative values of the Na^+ and K^+ permea

bilities of such a nature as to permit first one and then the other emf to dominate the transmembrane potential

The membrane of the resting fiber is characterized by a rather low ionic permeability It is, however, more permeable to K^+ ions than to Na^+ ions, and therefore the transmembrane potential is determined by E_K, the emf associated with the K^+ gradient

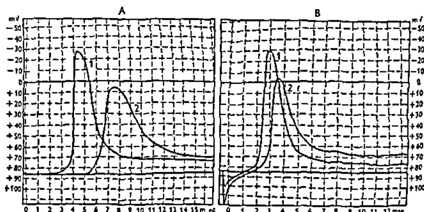


FIG 2 9 The action of low external Na^+ on the action potential of a skeletal muscle fiber of the frog (*A*) Normal (1) and 20 per cent of normal (2) Na^+ concentration (*B*) Normal (1) and 30 per cent of normal (2) Na^+ concentration (*From original of Fig 13 W L Nastuk and A L Hodgkin J Cell Comp Physiol* 35 39–73 1950)

Depolarization of the membrane increases the permeability of the membrane to Na^+ thereby permitting a certain influx of Na^+ ions and further depolarization The greater the depolarization of the membrane the greater the permeability to Na^+ becomes and the more marked is the contribution of E_N to the transmembrane potential This change progresses to the state of the fully active membrane in which the Na^+ permeability is higher than the K^+ permeability and the transmembrane potential is dominated by E_N At this time the state of the membrane may be represented by Fig 2-8*B*

It is obvious that the membrane must undergo further permeability changes to return to its resting state The nature of repolarization in cardiac fibers is obscure and the theory developed for the giant axon of the squid can apply only with modification, if at all Nevertheless the mechanism of repolarization in the giant axon

will be described here for the same reason that the mechanism of depolarization was, namely, the influence of the theory on the formation of theories and experiments on cardiac tissues

The fully active giant axon reverts toward its resting state through changes in both Na^+ and K^+ permeability The initial change is a decrease in Na^+ permeability, which is known as *inactivation* (Hodgkin and Huxley 1952*a*) In essence, the increase in Na^+ permeability which results from depolarization is self limiting and transitory The general phenomenon of inactivation is discussed below The main point here is that very soon after the peak of the action potential P_N begins to fall toward its resting value As a result of this the transmembrane potential begins to return toward the resting level, since the fall in P_N leads to a relative increase in the contribution of E_K to the transmembrane potential This fall in P_N toward the resting value would in itself suffice to return the transmembrane potential to its resting value However another important change aids in repolarization This change is a delayed increase in K^+ permeability Somewhat after the beginning of the fall in P_N P_K begins to rise Both the rise and the delay in the rise in P_K have been shown to result from depolarization, i e depolarization is in itself enough to cause P_K to rise but only after a delay The combined effect of a falling P_N and a rising P_K is of course to increase the contribution of E_K to the transmembrane potential and to repolarize or even temporarily to hyperpolarize the fiber

Inactivation of P_N Depolarization produces an initial increase in P_N and then a decrease The decrease plays a role in the repolarization phase of an action potential but also has a wider significance Any depolarization, however small, produces some increase in P_N followed by inactivation Moreover, inactivation is not simply a return of P_N to the value it had before the depolarization It involves, as the term implies a process which reduces the ability of the membrane to increase its P_N in response to a second depolarization The ability of the membrane to develop regenerative depolarization is therefore inactivated This may be indicated schematically by the equation

$$\text{Available } Na^+ \text{ carrier} + \text{depolarization} \rightarrow \text{active } Na^+ \text{ carrier} \rightarrow \text{inactivated } Na^+ \text{ carrier}$$

If the fiber remains depolarized, the Na^+ carrier will remain inactivated. If the fiber is repolarized, it is found that

$$\text{Inactivated } Na^+ \text{ carrier} + \text{repolarization} + \text{time} \rightarrow \text{available } Na^+ \text{ carrier}$$

Time is inserted in this equation to indicate that recovery of the ability of the membrane to increase its P_N after a depolarization is slow compared with the initial increase in P_N which results from depolarization.

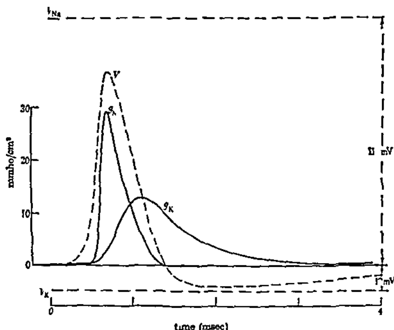


FIG 2 10 The action potential V and the Na^+ and K^+ permeabilities g_N and g_K of the giant axon of the squid (*Hodgkin 1957*). See text for discussion. (*Reproduced by permission of The Royal Society.*)

The inactivation of P_N is important in repolarization because inactivation contributes to the inability of the membrane to respond with a new depolarization during the repolarization phase. That is, inactivation contributes to refractoriness; the other cause of refractoriness is the high P_K during repolarization. The high P_K tends to hold the membrane near E_K and makes it more difficult for a cath

odal stimulus to depolarize the fiber while the inactivation of P_{Na} means that, even if the fiber is depolarized its ability to develop regenerative depolarization will be reduced

Since the rate of rise of the action potential as well as its amplitude depend on the ability of the membrane to increase P_{Na} anything which causes inactivation will diminish both the rate of rise and the amplitude of the action potential The most commonly observed cause of inactivation is a lowered resting potential Depolarization caused by high external K^+, for example will cause inactivation, as will depolarization secondary to anoxia The rise and fall of the Na^+ and K^+ permeabilities in the squid giant axon is shown in Fig 2-10 It has been shown (Hodgkin and Huxley 1952*b*) that the sequence of *permeability* changes shown in Fig 2 10 is adequate to explain the shape of the propagated action potential of the squid giant axon

Some Consequences of the Ionic Theory

Certain of the consequences of the Hodgkin theory or of any ionic theory of the action potential which will be referred to in the following chapters are discussed briefly here

Permeability Change and Membrane Impedance If the crucial event in the development of active depolarization is an increase in ionic permeability then since ions can move more freely across the membrane the electrical impedance of the fiber should fall during the upstroke of the action potential This has been found to be true in the giant axon and also in Purkinje fibers (see Fig 7-12)

Active Transport of Ions If there is any permeability to Na^+ when the fiber is at rest Na^+ will tend to enter the fiber slowly Furthermore if the permeability to Na^+ increases during activity Na^+ will enter in greater quantities when the fiber is active

Since the intracellular concentration of Na^+ remains low in spite of these two sources of Na^+ influx it seems probable that some mechanism exists which actively extrudes Na^+ from the cells Since this extrusion is against the gradient of concentration and of electric field both of which tend to push Na^+ into the cell it seems probable that metabolic energy is required for Na^+ extrusion Efforts have been made to study the rate of Na^+ entry into cardiac cells during activity and also to study the effects of metabolic inhibitors on the Na^+ distribution

If repolarization is associated with an increased permeability to K^+, it should also be associated with a flow of K^+ out of the fiber Also there is a loss of fiber K^+ across the resting membrane Studies which have been made with tracer K^+ on atrium and ventricle are discussed in Chaps 3 and 4

Threshold Although the Hodgkin theory does not predict the existence of a "threshold" in a mathematically rigorous sense (Fitzhugh, 1955), there is a point at which Na^+ permeability begins to increase very steeply as a function of depolarization On the other hand since any depolarization results in some increase in Na^+ permeability and therefore in some regenerativeness it is expected that active subthreshold depolarizations or local responses can be seen Such local responses are seen in cardiac muscle and are discussed in Chap 8

Recapitulation

The theory advanced by Hodgkin to explain the action potential of the giant axon of the squid has exerted great influence on the study of the cardiac action potential This theory holds that the transmembrane potential of resting and active fibers may be explained by the presence of two sources of emf which alternately dominate the transmembrane potential One, the emf resulting from the K^+ concentration gradient across the membrane, dominates the transmembrane potential of the resting fiber For this reason the transmembrane potential of the resting fiber can readily be changed by changing the extracellular K^+ concentration The other, the emf resulting from the Na^+ concentration gradient across the membrane, gives rise to an emf of opposite polarity to that caused by the K^+ gradient This is so because the Na^+ concentration gradient is oppositely directed to the K^+ concentration gradient

The change in transmembrane potential associated with activity does not come about because of a change in either of the two emf's but because of a change in ionic permeability The resting fiber is more permeable to K^+ than to Na^+ A small depolarization of the resting fiber increases the Na^+ permeability and thus increases the contribution of the Na^+ emf to the transmembrane potential Since increasing the contribution of the Na^+ emf increases the depolarization the fiber possesses a mechanism by which depolarization is

regenerative Once the Na^+ permeability reaches its maximum, it is considerably greater than the K^+ permeability

The fiber would remain depolarized if the Na^+ permeability remained greater than the K^+ permeability Two factors cooperate in returning the fiber to its resting state One is the decrease in Na^+ permeability This decrease depends on the fact that depolarization of the membrane tends to reduce by inactivation the high Na^+ permeability which has resulted from that same depolarization The cycle of depolarization → increased Na^+ permeability → increased depolarization is thus rendered self limiting by the fact that depolarization causes inactivation and decreased Na^+ permeability Repolarization also depends upon the fact that depolarization causes an increase in K^+ permeability The action potential thus results from a series of interactions between transmembrane potential and ionic permeabilities It will be seen in the following chapters that this theory has influenced attempts to interpret the diastolic depolarization characteristic of pacemaker fibers, the upstroke of the action potential the slow rise of the action potential seen in fibers of the atrioventricular node, the effect of acetylcholine on atrial action potentials and the process of repolarization in all cardiac fibers

3

THE ATRIUM

The muscle of the atrium is often thought to be a functionally homogeneous tissue which possesses only gross anatomical specializations. Futhermore, preparations of atrial muscle from different species are usually treated as though their properties were the same. Neither of these assumptions is correct. The atrium contains the sinoatrial and atrioventricular nodes, residua of the sinus venosus and the sinoventricular canal, and remnants of embryonal structures such as the venous valves (Patten, 1956). All these reveal important functional differences from ordinary atrial muscle and show distinct electrophysiological characteristics (Paes de Carvalho, de Mello, and Hoffman, 1959). Records of the electrical activity of single fibers have emphasized differences between the atrial muscle of amphibia and mammals as well as between different fiber groups in a single atrium. In this chapter we shall point out these differences and indicate their importance to an understanding of the origin and spread of activity. The sinoatrial and atrioventricular nodes are treated in separate chapters.

THE ATRIAL ACTION POTENTIAL

The Typical Contractile Fiber

Configuration. A typical transmembrane action potential recorded from a fiber belonging to the main mass of contractile fibers in a mammalian atrium is shown in Fig. 3.1. There is a constant level of membrane potential during phase 4, an abrupt onset of rapid depolarization, and a prominent reversal. Repolarization proceeds with

only minor changes in velocity so that the action potential is more or less triangular in shape In all fibers a rapid phase of repolarization (phase 1) can be distinguished, in some phase 2 is prominent and assumes the appearance of a plateau, while in others the recovery limb of the action potential is concave upward and separate

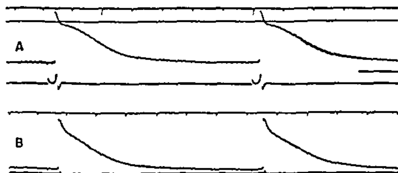


FIG 3-1 Transmembrane action potentials and electrograms recorded from dog atrium Top trace in A and B shows time marks at intervals of 100 and 500 msec Second trace in A is line of zero potential Horizontal bar at right of A represents minus 100 mv Bottom trace shows unipolar electrogram in A and bipolar electrogram in B Large driving stimulus artifact precedes electrogram complex in A

phases 2 and 3 cannot be identified In all records however the terminal phase of repolarization is characterized by an extremely slow return of the transmembrane potential to the resting value Action potentials of this sort have been found in the atria of dogs (Hoffman and Suckling 1952) cats (Hoffman and Suckling 1952) rabbits (West 1955a) and rats (Webb and Hollander 1956a) The precise shape of the recovery limb is variable under physiological conditions such as minor changes in ionic concentration and the presence of autonomic mediators Thus under different conditions the action potential of the same fiber may show a convex or a concave recovery limb The specialized atrial fibers show distinctive action potentials which are discussed below

The action potentials of atrial fibers of frogs and turtles are markedly different in configuration from those seen in mammalian atria In the atria of frogs and turtles the action potential of the single fiber shows a somewhat slow upstroke followed by a distinct and prolonged plateau A rapid initial phase of repolarization is not

TABLE 3-1 RESTING- AND ACTION POTENTIAL AMPLITUDE AND DURATION IN VARIOUS SPECIES

Species	Tissue	Fiber diameter μ	Technique	Resting potential mv	Action potential mv	Duration † msec	Source
Dog	Ventricle	16	IS*	80 (65–95)	100 (80–120)	200–300	Hoffman and Suckling 1952
		16	IS	82 (76–96)	102 (96–114)		Trautwein and Zink 1952
	Auricle	10	IS	85 (68–94)	100 (82–118)	200–300	Hoffman and Suckling 1952
	Papillary muscle	16	IV	85 (70–95)	105 (82–125)	200–300	Hoffman and Suckling 1953
	Auricle	10	IV	85 (68–95)	105 (82–120)	200–300	Hoffman and Suckling 1953
	Purkinje fiber	30	IV	96 (92–99)	121 (116–138)	250–300	Trautwein and Zink 1952
	Purkinje fiber	30	IV	90 (73–104)	121	400	Draper and Weidmann 1951
Cat	Papillary muscle	16	IV	88	116	150–200	Trautwein Gottstein and Dudel 1954
	Auricle	10	IV	60 (35–85)	65 (30–95)	200–250	Burgen and Terroux 1953a
Kid	Purkinje fiber	75	IV	94	135	300–500	Draper and Weidmann 1951
Calf sheep	Purkinje fiber	60	IV	98 (91–110)	132	500	Weidmann 1955b

Rat	Ventricle Auricle		IV IV	90 62	120 75 2	50–90 61	Matsuda and Hoffman unpublished Hollander and Webb 1955
Rabbit	Auricle		IV	78	92	150–200	West 1955a
Frog	Ventricle	10	IS IV	58 4 (40–80)	74 2 (50–110)	1 000	Kleinfeld Stein and Meyers 1954
		10	IS IV	64 5 (27–112)	77 2 (30–132)		Woodbury Hecht and Christopherson 1951
		10	IS	81 5 (76–89)	102 5 (95–112)	500–600	Ware et al 1957
Chick embryo	Ventricle		IS	39 3 (10–70)	53 5 (13–100)	100	Fingl et al 1952
	Auricle		IS	29 2 (10–41)	39 2 (11–81)	50–100	Fingl et al 1952
Turtle	Ventricle		IV IS	85 66 (54–86)	110 76 (60–100)	 600–1 400	Weidmann personal communication Sano et al 1956
	Auricle	30–80	IS	56 (50–63)	65 (55–90)	400–700	Sano et al 1956
Guinea pig	Ventricle		IV	52 8	67 2	75–100	Johnson 1956

* IS in situ IV in vitro
† Action potential duration varies with rate and temperature the values in this table at best give the order of magnitude

prominent at the temperatures usually employed, but separate phases 2 and 3 are clearly marked The transmembrane potential is usually quite constant during phase 4

Magnitude In dogs cats, rabbits, and rats the resting potential ranges from 80 to 90 mv and the action potential from 100 to 120 mv (Table 3 1) Lower values recorded by some investigators probably result from inadequate experimental techniques such as the use of microelectrodes of too great a tip diameter Experiments on intact atria in situ have given values for resting and action potentials similar to those cited for in vitro preparations (Hoffman and Suckling 1952) In both instances it is likely that the upper end of the normal range approximates the true magnitude of transmembrane potentials It is possible, however that a considerable range of values may be found in a normal atrium Thus various authors have found that both resting and action potential magnitudes are distributed on a rough bell curve (Ware et al 1957 Burgen and Terroux, 1953*a*) The problem of whether the random variation described by this curve results from physiological or experimental variation is unsolved In all probability there is a physiological variation but it may well be much narrower in range than the experimental observations suggest

Early studies reported low values for the resting potential and action potential of single atrial and ventricular fibers of amphibian hearts (Woodbury et al 1951, Kleinfeld et al 1954) but recent work indicates that values for ventricle are quite similar to those reported for mammalian tissues (Ware et al 1957) The fibers of the amphibian atrium are small in diameter and electrode size is crucial in studying their resting and action potentials It is not therefore unreasonable to suppose that studies with sufficiently fine electrodes will reveal resting and action potentials comparable in magnitude to those seen in mammalian atria

Rate of Depolarization The rising velocity of the atrial action potential has been adequately studied in only a few experiments The maximum rate of depolarization reported in dog and rabbit fibers is at least 80 volts/sec Amphibian fibers show lower rates of depolarization

Duration The duration of the atrial action potential varies somewhat with heart rate At comparable rates however the duration in large mammals such as dog and cat is greater than in small mam-

mals such as the rat (see Table 3 1) At a rate between 100 and 150 beats per minute the average duration in the larger mammals, at 37 to 38°C would be from 200 to 250 msec and in the smaller mam mals at 30°C, from 50 to 70 msec (Hollander and Webb, 1955) The duration of amphibian atrial potentials is long compared with those of mammalian atria even when the temperature and rate are com parable Kleinfeld (personal communication) has noted that action potentials recorded from the right atrium of guinea pig hearts con sistently show a longer duration than those recorded from the left atrium

Conduction Velocity The conduction velocity of mammalian atrial muscle is somewhat difficult to measure because of the syncytial nature of the tissue and the numerous interconnections between bundles of fibers Values of 0 8 m/ ec for dog atrium (Brendel et al 1950) and of 0 45 to 0 6 m/sec for rabbit atrium (Paes de Carvalho et al 1959) have been reported our own studies of dog atrium sug gest that the maximum velocity may be as high as 1 0 m/sec in intact atria as well as in isolated preparations of atrial muscle Atrial fibers possessing specialized characteristics have not been shown to conduct at a higher velocity than plain muscle fibers (Paes de Carvalho et al 1959), on the contrary in many instances the spread of activity is greatly slowed in certain of these tissues (see below)

Other Atrial Fibers

The atrial action potential described above is recorded from the contractile tissue which makes up the mass of the atrium Recent studies of rabbit atrium (Paes de Carvalho et al 1959) have demonstrated the existence of several other groups of fibers of con sistent anatomical location the action potentials of which are quite distinct in configuration (see Fig 1 2) Surrounding the junction of venous tissue with atrial muscle there is in the rabbit heart a fine bundle of fibers which has been called the *sinoatrial ring bundle* Action potentials recorded from single fibers of this bundle sugges tively resemble those recorded from Purkinje fibers in the mam-malian ventricle, the initial upstroke of the action potential is fol-lowed by a brief rapid repolarization (phase 1) which carries the transmembrane potential back approximately to the zero line Subsequently there is a clear plateau and fairly rapid repolarization

during phase 3 In addition, many fibers of the sinoatrial ring bundle show a slow depolarization during phase 4 Action potentials *recorded from fibers of the sinoatrial ring bundle differ from those of* Purkinje fibers, however in that the rising velocity of the action potential is lower than that of Purkinje fibers, the duration of the plateau is less, and the conduction velocity in these fibers seems to be the same as in other areas of the atrium, i e, 0 8 to 1 0 m/sec

The functional significance of these fibers has not yet been clearly demonstrated However two major possibilities have been considered It seems reasonably certain (see Chap 5) that if the pacemaker activity of the sinoatrial node is depressed by acetylcholine or excess K^+ the pacemaker site may shift to these specialized atrial fibers Moreover the embryologic origin and anatomical distribution of part of the sinoatrial ring bundle suggest that it might act as a specialized atrionodal conduction path Under normal conditions (Eyster and Meek 1914, 1916 Hoffman and Paes de Carvalho, unpublished observations) interruption of this structure fails to prolong the atrioventricular conduction time if the terminal portion of the crista terminalis is intact However, recent studies (de Mello, unpublished observations) suggest that when conduction through plain atrial muscle is depressed as by excess K^+, the major link between the atrium and atrioventricular node is through the specialized atrial fibers

A second group of fibers which is found in the rabbit atrium and which possesses characteristic electrical properties is located around the entire circumference of the atrioventricular valve These fibers, which may represent a residuum of the primitive sinoventricular canal conduct at a very low velocity (approximately 0 2 m/sec) and show some slow depolarization during diastole The action potential is similar to that recorded from fibers in the atrial end of the atrioventricular node (see Chap 6) in that the rising velocity is extremely low the resting potential and action potential are smaller than those recorded from ordinary atrial muscle, and the upstroke of the action potential is often slurred or notched Latent pacemaker activity may be particularly marked in fibers of this type which are located close to the base of the pulmonary artery It has been postulated that it is fibers of this type in the upper end of the atrioventricular node which are responsible for most of the atrioventricular delay

If careful attention is paid to the shape of the action potential, three additional fiber groups can be identified (Paes de Carvalho et al, 1959) These fibers are located in the crista terminalis the pectinate muscles and the atrial roof In general, action potentials from the crista terminalis show a clear plateau, while action potentials from atrial roof fibers most frequently show an upward concavity of the repolarization limb The rising velocity of the action potential is greatest in the fibers of the atrial wall between the pectinate muscles Preliminary studies show that these several fiber groups may respond in different ways to a variety of agents such as acetylcholine and epinephrine, however their presence in the atria of other mammals has not yet been demonstrated and their functional importance has not been ascertained It is likely, however, that the different shapes of atrial action potentials noted by all investigators in the preparations of atrial muscle they have employed are due in part at least to the presence of such distinctive groups of fibers It has not yet been possible to correlate action potential shape with the histologic structure of certain atrial fibers such as those described by Todd (1932) and by Robb (personal communication)

THE ELECTROGRAM OF ATRIAL MUSCLE

The electrogram of atrial muscle whether recorded with unipolar or bipolar leads is similar to that of ventricular tissue with one major exception Just as an atrial T wave is seldom recorded in standard electrocardiographic tracings the repolarization of atrial fibers is often not clearly recognized in the atrial electrogram However, unipolar tracings recorded with the active electrode in close proximity to the muscle show a distinct deflection which has its maximum amplitude during rapid repolarization of the fibers close to the active lead (Fig 3 5 4) These records obtained from isolated preparations of atrial tissue are similar to those obtained from a lead located in the cavity of the intact atrium (Kossmann et al, 1950) and clearly show that the atrial T wave commences immediately after the depolarization of a given area The causal relationship between the time and rate of repolarization of the atrial muscle fibers and the time and amplitude of the electrogram deflection designated as the T wave is clearly shown in the records obtained after the addition of acetylcholine (Fig 3-5)

It is incorrect therefore, to assume that the atrial *T* wave is not seen in the usual limb lead electrocardiogram because it is obscured by ventricular depolarization Repolarization of some atrial fibers commences immediately after the initial deflection of the *P* wave and continues throughout all the *PR* interval The lack of prominence of the atrial T wave in the surface leads results rather from the asynchronous activation of the atrial tissue the spatial distribution of the atrial muscle with respect to the recording electrodes, and the rather gradual repolarization of the individual atrial fibers In conditions associated with rapid repolarization of the atria, such as flutter the atrial T wave is clearly seen in certain of the limb leads (Prinzmetal et al , 1952) Records obtained from frog and turtle are of some interest in this respect In these hearts the transmembrane action potential of atrial muscle shows a clear plateau and rapid repolarization during phase 3 the atrial electrogram therefore records a classical *T* wave after an isoelectric *ST* segment This atrial T wave which may be seen in surface leads during atrioventricular dissociation actually is concealed during normal cardiac activity by the complexes resulting from ventricular depolarization

EFFECTS OF PHYSIOLOGICAL VARIABLES ON THE ATRIAL ACTION POTENTIAL

One of the more interesting aspects of the electrical activity of single cardiac fibers is the observation that under conditions which might be described as normal the time course of depolarization remains relatively fixed while the course of recovery shows marked changes in response to a variety of physiological factors Even if both depolarization and repolarization are altered by some single factor such as temperature the magnitude of change in the recovery processes is considerably more prominent Of added interest is the fact that, while the change in depolarization caused by a given agent is most often the same in fibers from any tissue of the heart, in repolarization we find marked differences in response between fibers from atrium ventricle, and specialized tissues

Rate and Rhythm

Factors such as altered temperature autonomic mediators, or changed ionic concentration which cause changes in the frequency

and rhythmicity of the contraction of cardiac muscle also influence the shape of the action potential of the cardiac fiber It is therefore difficult to study the effects of changes in rate brought about by any means other than the use of artificial driving stimuli to initiate each contraction Excessive changes in the frequency of contraction of the intact heart may result in alterations of blood flow or oxygen supply which will act upon the individual cardiac fibers and induce changes that are not directly caused by the heart rate Most of these difficulties can be overcome in studies of an isolated preparation of cardiac muscle which is supported in a tissue bath and driven by electrical stimuli In such a preparation the relationship between the frequency of contraction and the electrical activity of the individual fiber can be demonstrated with reasonable ease

In atrial muscle as shown in Fig 3 2 the most prominent effect of an increase in the frequency of contraction is a decrease in action-potential duration This change does not appear in the atria of dogs and cats until the heart rate approaches 60 to 100 beats per minute When the repetition rate of the action potential is so high that depolarization begins before the preceding repolarization is quite complete not only is the action potential duration further decreased, but also the rising velocity and amplitude of the upstroke are diminished This latter change is due primarily to the low membrane potential present at the time of depolarization and not to some cumulative effect of the high rate as can be seen from records of single premature beats (Fig 3 3) In contrast transmembrane potentials of rat atrium show little decrease in duration at high rates (Hollander and Webb 1955) As with rapid rate when an extrasystole arises from a partially repolarized fiber the duration upstroke velocity and amplitude of the action potential are all decreased Weidmann (1955*a*) has shown that the changes seen in the premature action potential are similar to those induced by partial depolarization of the resting fiber In addition when the rate of repolarization of the atrial fibers is increased by acetylcholine, the amplitude and rising velocity of the action potential are maintained near normal at rates far in excess of those shown in Fig 3 2 (Hoffman and Suckling 1953)

The rate induced change in action potential duration is of considerable interest in that a similar effect is not encountered in other excitable tissues on the contrary in frog nerve rapid activity

fiber of the intact dog atrium (Hoffman and Suckling 1953) show that this change is primarily in the time course of repolarization With weak vagal activity the recovery limb of the action potential develops a marked upward concavity (Fig 3-4), stronger vagal stimulation greatly accelerates all phases of repolarization, so that the duration of the action potential is decreased by as much as

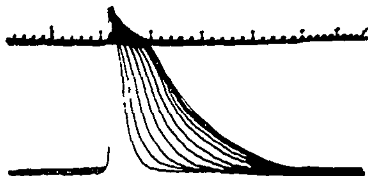


FIG 3-4 Transmembrane action potentials recorded from a single fiber of in situ dog atrium prior to and during vagal stimulation Top trace is line of zero potential and shows time marks at intervals of 10 and 50 msec Note progressive decrease in the duration of the action potential and absence of appreciable changes in resting potential or reversal (*Hoffman and Suckling* 1953)

200 msec This effect is also seen in monophasic records from turtle atrium (Churney et al , 1949) and dog atrium (Schütz 1931, Hoffman, Siebens and Brooks, 1952) obtained by recording between injured and normal tissue For the full effect of vagal activity to develop requires 2 to 4 sec, while recovery takes several times as long Such records obtained from the in situ dog auricle reveal two additional minor changes There is a slight increase in conduction velocity and a variable small increase in the resting potential

When acetylcholine is added to isolated preparations of mammalian atrium the action potential of unspecialized fibers is altered in a manner similar to that just described (Fig 3 5), thus in atria of dogs cats rabbits and rats the primary change is in the rate of repolarization As a result of accelerated repolarization the action-potential duration is decreased The action potentials recorded from atrial or sinus tissue of frog and turtle hearts are also shortened by vagal stimulation and acetylcholine (Castillo and Katz, 1955 Hutter and Trautwein 1955*a* and *b* 1956) In these atria the first

change is a decrease in the duration of the plateau, subsequently there is a complete disappearance of this phase of the action potential so that the record is indistinguishable from one obtained from mammalian atrium

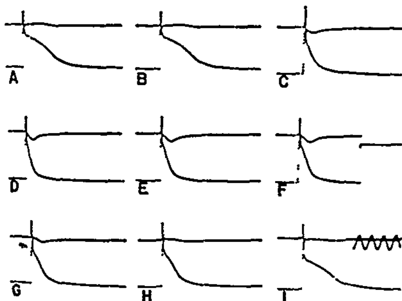


FIG 3-5 The effect of acetylcholine chloride 1 2 000 000 on the unipolar electrogram (top trace) and transmembrane action potential (bottom trace) of an isolated preparation of dog atrium (*A*) Control (*B*) through (*I*) onset of acetylcholine effect and partial recovery Voltage step in *F* +100 mv time calibration in *I* 20 cps Note simultaneous change in repolarization of single fiber and in atrial T wave Upstrokes of transmembrane action potentials are intensified by dotted lines

In the records shown in Figs 3-4 and 3 5 vagal stimulation and acetylcholine had little effect on the resting transmembrane potential If the initial value of the resting potential is low however as it may be under a variety of experimental conditions vagal stimulation or acetylcholine cause a fairly marked hyperpolarization Conversely if the resting potential is close to the K^+ equilibrium potential predicted for the tissue under study (see Chap 2) acetylcholine even in high concentration causes no change in this value (Trautwein and Dudel 1958*a*) This finding explains differing

results obtained in studies of the effect of acetylcholine on dog atrium (Hoffman and Suckling, 1953) and cat atrium (Burgen and Terroux, 1953*b*) and perhaps also the contrast between the change in resting potential of frog sinus (Hutter and Trautwein, 1956) and rabbit sinoatrial node caused by this agent (West, Falk, and Cervoni, 1956, Hoffman, unpublished)

The upstroke of the action potential of ordinary atrial fibers is largely unchanged by acetylcholine unless there is a simultaneous increase in the resting potential, in which case rising velocity is augmented and reversal increased *In contrast to this finding,* certain fibers in cat and rabbit atrium show a considerable decrease both in the rising velocity and amplitude of the action potential in the presence of high concentrations of acetylcholine This effect is particularly prominent in the specialized fibers of the rabbit atrium (Hoffman, unpublished) and is also seen in turtle and frog atrium during strong vagal stimulation (Hutter and Trautwein, 1956) and perhaps in all atrial muscle when the acetylcholine concentration is very high

The effects of acetylcholine on the repolarization of atrial fibers provide an excellent example of the relationship between the rate of change of membrane potential during repolarization and the amplitude of the T wave of the electrogram This relationship is shown in Fig 3 5 in which the records on the upper trace were obtained with a unipolar surface lead and those on the lower trace with an intracellular microelectrode Under control conditions the slow repolarization of the atrial fibers is reflected in a barely visible displacement of the base line of the electrogram simultaneous with phase 3 of repolarization After the addition of acetylcholine the repolarization of the atrial fibers is greatly accelerated, and there is a marked change in the atrial T wave which is inverted and increased in amplitude and appears earlier after the depolarization complex Since in this preparation acetylcholine causes only a slight increase in conduction velocity, the change in the atrial T wave must result from the altered rate of repolarization of the muscle fibers

An increase in K^+ permeability as the probable mode of action of acetylcholine on pacemaker tissues is discussed in Chap 5 The effects of acetylcholine on atrial fibers could be brought about by a similar increase in the permeability of the membrane to K^+ The

experiments of Burgen and Terroux (1953b) and of Trautwein and Dudel (1958a) have shown that when the resting potential of the atrial fiber is increased or decreased by changing the extracellular K^+ concentration, the action of acetylcholine is to bring the transmembrane potential closer to the K^+ equilibrium potential. Also, acetylcholine causes less acceleration of repolarization when extracellular K^+ is high than when the concentration of this ion is low (Hoffman, unpublished). In addition, acetylcholine has been shown to decrease membrane resistance (Trautwein, Kuffler, and Edwards, 1956) and to increase the flux of K^+ across atrial fibers (Holland et al. 1952a and b) and sinus fibers (Harris and Hutter 1956). An increase in K^+ permeability would alter the resting potential by increasing the contribution to this potential from the K^+ concentration gradient. Accelerated repolarization would similarly result from an enchanced efflux of K^+ during recovery. Finally, a decrease in the rising velocity and amplitude of the action potential upstroke would occur if the change in K^+ permeability of the resting membrane were great enough to make an appreciable contribution to the membrane potential during the period of increased Na^+ permeability which follows excitation.

Epinephrine and Norepinephrine

The effects of epinephrine on the electrical activity of atrial muscle are not impressive. In agreement with results obtained from monophasic records of turtle atrium (Churney 1952) records of the transmembrane potential of single atrial fibers of both mammals and amphibia show little consistent change after the addition of either epinephrine or norepinephrine. In some instances the action-potential duration is slightly decreased; this effect has been recorded from preparations of dog and cat atria (Brooks et al. 1955). In other instances, as in rat (Webb and Hollander 1956a) or rabbit (Hoffman, unpublished), the action potential is prolonged by these same agents. In spontaneously active preparations such as that shown in Fig. 3.6 it is difficult to separate changes due to epinephrine from those associated with a change in heart rate. Significant alterations in either the resting potential, the velocity of depolarization, or the amplitude of the reversal have not been reported by most investigators but have been noted in one study (Dudel and Trautwein 1955). The effects of sympathometic amines on atrial muscle

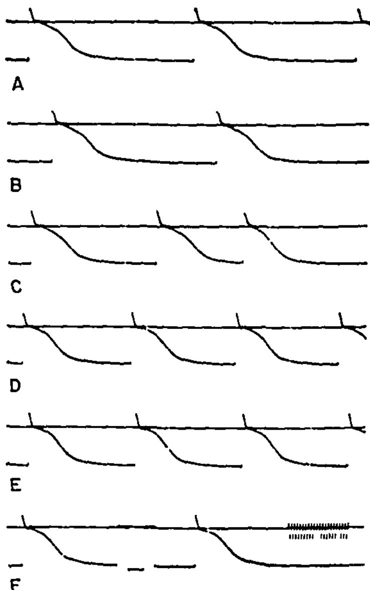


FIG 3-6 Segments of a continuous record showing the effect of *l*-epinephrine HCl 1 1 000 000 on transmembrane potentials of rabbit atrium Isolated preparation driven at a rate of 100 beats per minute (*A*) Control (*B*) through (*F*) show slight effects of epinephrine including extrasystoles (*C*) spontaneous activity at a rate of 160 beats per minute (*D*) and change in phases 2 and 3 of repolarization Time calibration 100 cps Voltage calibration in *F* is −100 mv

are thus much less dramatic than their actions on pacemaker tissues (see Chaps 6 and 7)

Ions

Sodium From the brief description of the events presumed to underlie the electrical activity of excitable cells given in Chap 2 it is obvious that any appreciable change in the extracellular Na^+

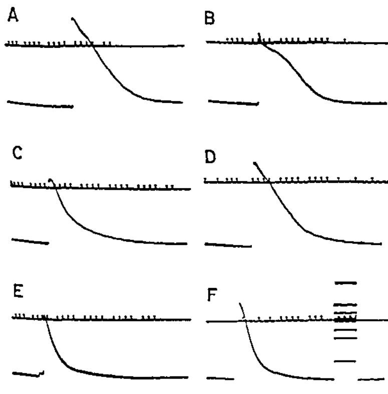


Fig 3-7 Transmembrane potentials recorded from single fibers of dog atrium showing the effects of decreasing the extracellular sodium concentration Top trace represents the line of zero potential and shows time calibrations at intervals of 10 and 50 msec Voltage calibration in *F* +50 +20 +10 −10 −20 −50 and −100 mv (*A*) Control (*B*) 50 per cent NaCl replaced by sucrose (*C*) 75 per cent NaCl replaced by sucrose (*D*) return to 100 per cent NaCl (*E*) 75 per cent NaCl replaced by choline chloride (*F*) return to 100 per cent NaCl All records obtained 25 to 30 min after changing solutions See text for discussion

concentration should have a predictable effect on the action potential of atrial muscle fibers Within a range of concentrations which permit the maintenance of excitability the effects of Na^+ on atrial muscle are in reasonable agreement with the predictions of the ionic hypothesis (Hodgkin, 1951) As can be seen in Figs 3 7 and 3-8,

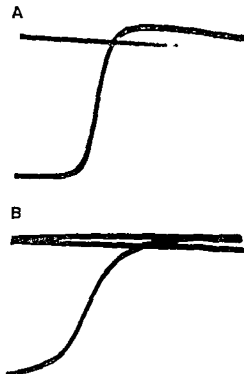


Fig 3-8 The effect of decreased extracellular Na^+ concentration on the rising velocity of the action potential of rabbit atrium (*A*) Normal Tyrode solution (*B*) Tyrode solution with 66 per cent of the NaCl replaced by an isoosmotic amount of sucrose See text for discussion

when more than half of the extracellular NaCl is replaced by an approximately isoosmotic amount of sucrose the transmembrane action potential of an atrial fiber shows a decrease in both amplitude and the maximum velocity of the upstroke If the extracellular NaCl concentration is decreased to 25 per cent of normal the rising velocity and reversal are further decreased In the particular experiments shown in Figs 3 7 and 3 8 the change in both parameters of the action potential is in approximate quantitative agreement

with the behavior of a membrane dependent largely on a sodium concentration gradient for its potential It can also be seen in the figure that the duration of the action potential is decreased in 25 per cent NaCl and the recovery limb has developed a marked upward concavity The possible mechanism responsible for this accelerated repolarization will be discussed in Chap 9 At this time we will merely mention the possibility that if there is any appreciable inward Na^+ current after the end of the action potential upstroke, this current would delay the restitution of the normal resting potential A decrease in Na^+ concentration gradient would diminish this inward current, at any given potential and thus permit a more rapid restoration of the resting potential by the outward repolarizing current

The changes caused by decreasing the extracellular NaCl to 25 per cent are constant over a period of several hours and can be completely reversed by restoring the Na^+ concentration to normal (Fig 3-7) If the concentration of NaCl is lowered to 10 to 15 per cent of normal however excitability is lost completely When on the other hand the extracellular Na^+ is increased above normal by 25 or 50 per cent by adding NaCl there is little immediate change in either the amplitude or rising velocity of the action potential Since however, the high Na^+ solution is hypertonic apparent disagreement with the predictions made in Chap 2 may be the result of osmotic damage to the fibers or of shifts of water and other ions

Some investigators have employed choline chloride as a substitute for NaCl in experiments of this type When large quantities of choline are used to replace Na^+ in studies of atrial muscle the cholinergic effects of this substance are quite apparent Figure 3 7 shows that when 75 per cent of the extracellular NaCl is replaced by choline chloride, the decrease in rising velocity and amplitude of the action potential are more prominent than with sucrose replacement Similarly the acceleration of repolarization is more marked Some of these differences might be explained if choline acts in a manner similar to that postulated for acetylcholine that is if it increases K^+ permeability to abnormally high levels It is quite likely that this is the explanation for the differences observed

Potassium If the extracellular K^+ concentration is increased or decreased, the effect on electrical activity of the myocardial fiber

may result primarily from any one of three major variables or from a combination of them The change in the transmembrane action potential may be caused directly by the elevated or depressed K^+ concentration outside the fiber, it may come about because the intracellular K^+ level has changed as a result of the primary change in extracellular concentration, and, finally, a change in resting potential, resulting directly from the effect of K^+, may in turn influence the amplitude shape or duration of the action potential (Weidmann, 1955a) In addition to these major effects changes in the Ca^{++}/K^+ ratio (Hoffman and Suckling 1956) or changes in threshold or conduction velocity may be of some importance in determining the ultimate amplitude and shape of the transmembrane action potential The most extensive studies of the effects of K^+ on cardiac tissue have been made on ventricular muscle and isolated Purkinje fibers discussion of these many possible interrelationships will be confined largely to these tissues Here we will present only a description of certain changes in the atrial action potential caused by varying the extracellular K^+ concentration

The records shown in Figs 3 9 and 3 10 were obtained from a single fiber of dog atrium The changes in potential in these records are plotted as a function of time in Fig 3-11 to facilitate quantitative description Just before the first action potential in Fig 3 9*B* the K^+ concentration of the Tyrode solution was suddenly increased to 60 mM/l by adding concentrated KCl, the immediate effect was a rapid fall in the resting potential which reached a steady value of 20 mv after approximately 10 sec This drop in resting potential was accompanied by an abrupt change in the amplitude of the action potential and by a loss of excitability At the beginning of the trace shown in Fig 3 9*D* the K^+ concentration was returned to normal, the following records are part of a continuous trace and show a slow return of the resting potential to the control value (Fig 3 10*J*) When the resting potential attained a value of 55 mv, the driving stimuli elicited local responses which alternated with small, slowly propagated action potentials (Fig 3 9*F*) Subsequently as the resting potential increased the action potential showed a progressive rise in amplitude, until, at a resting potential of 60 mv, the overshoot reappeared With complete restoration of the resting potential the action potential approximated its usual amplitude and configuration (Fig 3-10*J*)

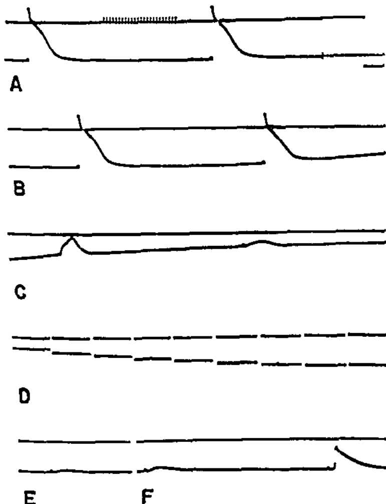


Fig 3 9 The effect of KCl on the electrical activity of dog atrium Transmembrane potentials recorded from the same single fiber from *A* through *F* Time calibration in *A* 100 cps voltage calibration 100 mv Top trace represents line of zero potential (Note drift indicated at time of withdrawal of electrode in Fig 3-10*K*) KCl added after first action potential in *A* Record is continuous from *A* through *C* KCl returned to normal concentration between *C* and *D* Segments in *D* are taken 10 30 50 75 105 150 210 270 and 330 sec after removal of KCl *E* is recorded 435 sec, and *F* 480 sec after returning to a normal concentration of KCl

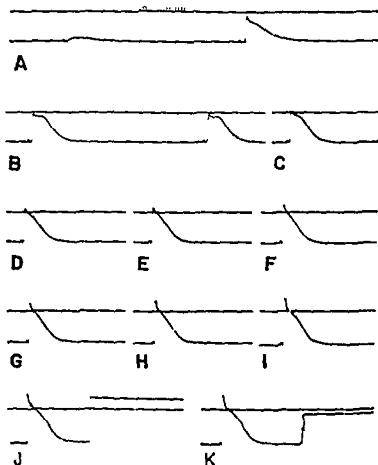


FIG 3-10 Continuation of the record shown in Fig 3 9 from the same fiber showing recovery from effects of excess KCl Records were obtained at the following times in seconds after return to normal solution (*A*) 490 (*B*) 520 (*C*) 535 (*D*) 550 (*E*) 565 (*F*) 500 (*G*) 630 (*H*) 735 (*I*) 855 (*J*) 915 and (*K*) 1 095 Note withdrawal of electrode at the end of K showing some drift Time and voltage calibrations are the same as in Fig 3 9 See text for discussion

The drop in resting potential following the sudden increase in extracellular K^+ concentration would be expected of a potential which depends largely on a K^+ concentration gradient across a semipermeable membrane (see Chap 2) If muscle from mammalian atrium is allowed to equilibrate with progressively higher concentrations of K^+, the change in resting potential is related to the

logarithm of the extracellular K^+ concentration in an approximately linear manner (Fig 3 12), although the slope of the line (35 to 55-mv change in resting potential for a ten fold increase in K^+) is somewhat less than that predicted by the Nernst equation On the

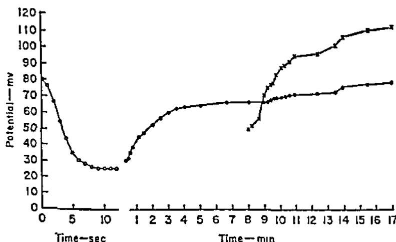


FIG 3-11 Graph of changes in the amplitude of the resting potential and action potential shown in Figs 3-9 and 3-10 o-o resting potential in excess KCl •—• resting potential and x x action potential after returning to normal solution Note rapid depolarization in high K^+ solution slower recovery of resting potential in normal Tyrode solution and relationship between magnitude of resting potential and action potential amplitude

other hand, if the K^+ concentration is decreased below normal or to zero, the resting potential of mammalian atrium usually fails to rise or actually falls (Fig 3 12) This departure from the predicted behavior probably results in part from a loss of intracellular K^+ since in a few experiments on rabbit hearts perfused through the coronary arteries (Lepeschkin and Hoffman, unpublished observations) an abrupt decrease to zero in the K^+ concentration of the perfusate was followed by a transient rise in resting potential to values close to 100 mv

Although the resting potential of atrial muscle behaves roughly in the manner expected of a K^+ diffusion potential several factors alter the quantitative aspects of the relationship between K^+ and transmembrane potential Burgen and Terroux (1953b) have shown that when atrial fibers are exposed to acetylcholine at any extra

cellular K^+ concentration the resting potential of the fibers is closer to the predicted K^+ equilibrium potential than in the absence of this agent. This action of acetylcholine would be expected if the permeability of the membrane to potassium were increased. The

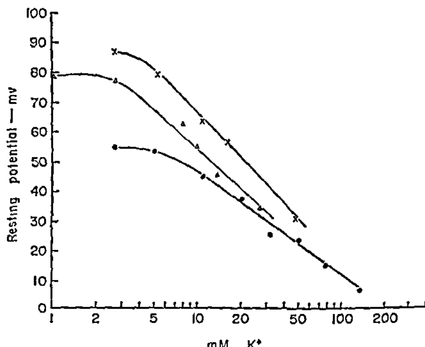


FIG. 3.12. Relationship between the concentration of extracellular potassium and the resting transmembrane potential of single fibers. Ordinate: resting potential in millivolts; abscissa: extracellular K^+ concentration in millimoles per liter. ●—● cat atrium (*data from Burgen and Terroux* 1953a); *x x* dog papillary muscle; Δ Δ rabbit atrium. Each point represents the average of three or more determinations. The extracellular Ca^{++} concentration for these experiments was as follows: ●—● 1.9; *x x* 2.7; Δ Δ 1.9 mM/l. (*Hoffman* 1959.)

extracellular Ca^{++} concentration also changes the relation between K_o^+ and resting potential. Low Ca^{++} increases the resting potential in low K^+ solutions and excess Ca^{++} limits the depolarization caused by high K^+ (Hoffman and Suckling 1956).

The changes in the amplitude of the action potential shown in Figs. 3.9 and 3.10, as well as the loss of excitability, are almost certainly caused by the low resting potential and not by any other effect of K^+. The effect of resting potential on the regenerative

response and excitability is discussed in Chaps 7 and 9, at this time it is sufficient to mention two sets of observations First, in studies of mammalian Purkinje fibers Weidmann (1955a) found that electrical repolarization of a fiber depolarized by K^+ excess restored the rising velocity and amplitude of the action potential to normal Second any factor which causes a decrease in resting potential also decreases the amplitude of the action potential The alterations in the shape and duration of the action potential seen in Figs 3 9 and 3 10 are somewhat more difficult to account for Most workers, using a variety of hearts have concluded that excess K^+ shortens the duration of the action potential Conversely low K^+ has been shown to accelerate at least the initial phases of repolarization (Surawicz et al 1959) In addition to these effects the low resting potential may be a direct cause of a slower rate of repolarization (Webb, 1956) Finally in intact hearts, changes in the extracellular K^+ level usually cause some change in heart rate, which in turn influences the time course of repolarization

The records of recovery from excess K^+ shown in Figs 3 9 and 3 10 reveal several points of interest when contrasted with those shown in Fig 3 13 In this latter experiment K^+ depolarization was followed by a return to Tyrode solution containing a normal amount of K^+ and an excess of Ca^{++} In the presence of a high concentration of extracellular Ca^{++} (Fig 3 13) the driving stimuli elicit local responses at a lower resting potential than in a solution containing a normal concentration of Ca^{++} (Fig 3 9) and the recovery of the resting potential is more rapid Also propagated action potentials appear at a lower value of resting potential in high Ca^{++} solution, and the amplitude of the action potential at any resting potential below normal, is increased All these effects of Ca^{++} are to be expected from the results obtained by Weidmann (1955b) which are described in Chap 7 and from those of Hoffman and Suckling (1956) In general, it can be said that excess Ca^{++} has had two major effects in this experiment One has been to increase the resting potential in the presence of an elevated concentration of K^+ and the other has been to enhance the ability of the fiber to show regenerative depolarization at low values of the resting potential

Calcium and Magnesium Several effects of Ca^{++} on the electrical activity of atrial muscle have just been described in conjunction with the discussion of K^+ If the resting potential of an atrial fiber

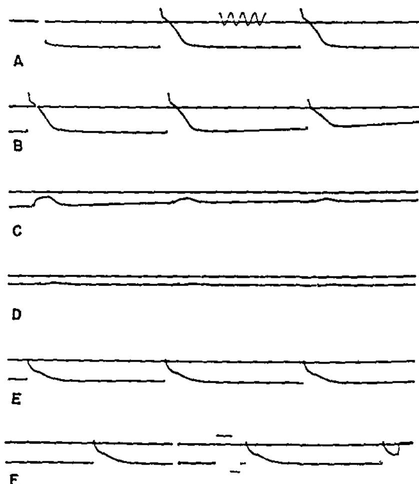


FIG 3 13 The effects of K^+ and Ca^{++} on the transmembrane potentials of a single atrial fiber in an isolated preparation of dog heart Top trace represents line of zero potential time calibration in *A* 10 cps Traces are interrupted every 500 msec Voltage calibration in *F* +100 mv KCl added to perfusion fluid after first action potential in *A* Solution containing normal K^+ and excess Ca^{++} added between *D* and *E* Note artifacts of driving stimuli and withdrawal of electrode at the end of *F* See text for discussion

is within the normal range, even a marked alteration in Ca^{++} concentration causes only a slight change in this measurement The same is true for the rising velocity and amplitude of the action potential, raising or lowering the concentration of Ca^{++} results in only a moderate increase or decrease in these values if the resting potential is close to 90 mv Neither marked excess (15 mM/1) nor complete depletion of Mg^{++} has been shown to noticeably change the resting potential or the amplitude of the action potential (Brooks et al 1955)

In contrast the concentration of Ca^{++} has a marked effect on the time course of repolarization Low levels of this ion in the extracellular fluid prolong phase 2 of the action potential If Ca^{++} and Mg^{++} are both markedly reduced, the atrial action potential assumes a shape identical to that of a typical ventricular fiber with phases 1 2, and 3 all clearly demarcated (Hoffman and Suckling 1956) The effects of Ca^{++} excess on repolarization are quite the opposite the initial phases are accelerated, and a slow terminal phase appears High concentrations of Mg^{++} have a similar, but less pronounced action

The effects of Ca^{++} on the excitability of atrial muscle fibers are quite similar to those described for other tissues (Brink 1954) The threshold stimulus requirement is increased by high and decreased by low levels of Ca^{++} and the responses of the partially refractory fiber are similarly enhanced or depressed (Weidmann, 1955*b* Hoffman and Suckling 1956) It is interesting that tissues from different parts of the same heart appear to have quite different sensitivity to Ca^{++} depletion Thus the isolated papillary muscle of dog or cat heart remains normally excitable for many hours in solution that is almost free of Ca^{++} while fibers from the atrium of the same hearts rapidly become inexcitable (Brooks et al 1955) Since the loss of excitability is not associated with an important change in the resting potential it probably results from the effects of Ca^{++} on mechanisms controlling the Na^{+} permeability of the membrane (Weidmann 1955*b*)

Chloride Only a limited number of studies of the effects on atrial muscle of changes in the Cl^{-} concentration have been done This problem is of interest in relation to the possible contribution of the Cl^{-} concentration gradient to the resting potential and to repolarization In terms of its distribution this ion could account for a major

part of the resting membrane potential, and under certain conditions it contributes to the transmembrane potentials of some tissues (Stampfli, personal communication, Coombs et al, 1955) In studies of isolated preparations of rabbit atrium (Hoffman, Paes de Carvalho, and de Mello unpublished) it has been shown that replacement of up to 75 per cent of extracellular NaCl by sodium acetate has no demonstrable effect on the amplitude of the resting potential or the rising velocity and amplitude of the action potential for periods up to several hours The voltage time course during recovery was similarly unaffected When Cl^- was replaced by NO_3^-, there was no immediate change in resting potential or action potential, after one half hour or longer however, the resting potential decreased and excitability was lost In those experiments replacement of Cl^- by NO_3^- did not cause a prolongation in the atrial action potential, as it does in fibers of skeletal muscle (Hill and MacPherson 1954)

Drugs and Other Agents

Carcolin and Mecholyl are indistinguishable from acetylcholine in their effects on the transmembrane potentials of rabbit atrium The shortening of the action potential produced by these agents is reversed by atropine and also by epinephrine (Marshall and Katsh, 1957) Neostigmine, on the other hand causes variable shortening of the atrial action potential as does strychnine (Marshall and Katsh 1957) Hypoxia, substrate depletion or addition of dinitrophenol in low concentration all produce a similar change in the transmembrane potentials of atrial fibers from rat hearts (Webb and Hollander 1956*b*) Alterations in the amplitude of the resting potential and action potential are small but a marked shortening of the action potential is observed in each instance in association with a decrease in the force of contraction Higher concentrations of DNP cause loss of excitability this effect is counteracted by ATP (de Mello, 1959) Addition of adenine nucleotides (ATP ADP, AMP, adenosine) to preparations of rat atrium (Webb and Hollander, 1956*b*) similarly causes only slight changes in the amplitude of the transmembrane potentials but markedly decreases action potential duration and force of contraction Adenine ribose, phosphate and pyrophosphate on the contrary cause no important change in the transmembrane action potential of the same prepara

tion Changes in resting tension sufficient to cause a major increase in the force of contraction do not produce any alteration in the transmembrane potentials recorded from fibers of rat atrium (Hollander and Webb, 1955) In the same tissue an inverse relationship between the magnitude of the resting potential and the duration of the action potential has been noted (Webb, 1956)

Several other agents have been shown to cause prominent alterations in the duration of the atrial action potential Ouabain slows repolarization of rabbit atrium (West personal communication) as does quinidine (West, 1955b) Fluoride (Hoffman unpublished) has an effect on dog atrium similar to that of acetylcholine, azide, on the other hand decreases both the amplitude and duration of the transmembrane action potential of rabbit atrium

Temperature

Cooling or heating atrial muscle has two major effects on its electrical activity One is to cause a change in rate, and the other,

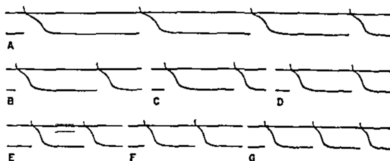


FIG 3 14 The effect of temperature on the transmembrane potentials of dog atrium Spontaneous rhythm Parts of a continuous record as temperature is increased from 22 (first action potential in *A*) to 32 C (*G*) Time calibration in *E* 50 cps Top trace line of zero potential

a change in the duration of the action potential Temperature also influences conduction velocity and excitability and the steepness of each of the several pha es of the action potential Several of these effects are seen in the records of transmembrane potentials of dog atrium shown in Fig 3 14 As the temperature of the tissue bath was quickly raised from 22 to 32°C the spontaneous rate of the preparation increased from 40 to 100 beats a minute, and the action

potential duration decreased from 500 to 250 msec Also clearly seen is a more distinct separation of the several phases of repolarization at the lower temperature, so that the action potential is quite similar in appearance to one recorded from a mammalian Purkinje fiber Similar effects of cooling on cat atrium have been reported by Burgen and Terroux (1953*a*) and for rat atrium by Hollander and Webb (1955) This clear separation is not present in records obtained from all atrial fibers, in some, cooling causes a smooth prolongation of the entire repolarization limb of the action potential and in others lowering temperature lengthens primarily the slow, terminal phase of repolarization The records shown in Fig 3 14 are chosen because they emphasize the different temperature sensitivities of the several phases of the atrial action potential The temperature coefficients for a tissue of the type shown in Fig 3 14 are as follows phase 1, −2 0 phase 2 −3 0, phase 3, −1 6, slow terminal phase of repolarization −1 6 These values are comparable to those obtained by Coraboeuf and Weidmann (1954) in a study of Purkinje fibers and demonstrate the greater temperature sensitivity of phase 2 If the tissue is driven at a constant rate, on the other hand the change in phase 2 is less marked, while the alterations in the other phases of the action potential are similar to those just described The importance of rate in studies of this sort is discussed in detail in Chap 4

In the records shown in Fig 3 14 there is no appreciable change in the amplitude of either the resting potential or the action potential, with more pronounced cooling however the resting potential is decreased and the action potential amplitude falls In cat and rabbit atrium conduction velocity is greatly slowed, and excitability is usually lost at temperatures below 20 to 23°C (Burgen and Terroux 1953*a* Marshall 1957) This loss of excitability is most directly related to the low resting potential since addition of acetylcholine presumably by increasing the K^+ permeability of the membrane raises the resting potential and restores propagation at even lower temperatures (Marshall 1957) These effects of changes in temperature on atrial muscle should be compared with changes in the electrical activity of ventricle (Chap 4) and Purkinje fibers (Chap 7) Additional discussion of temperature effects will be found in those chapters and also in Chap 9

PASSIVE ELECTRICAL PROPERTIES

Small bundles of frog atrial fibers have been used in a study of their membrane characteristics both under normal conditions and during the action of cholinergic drugs (Trautwein, Kuffler, and Edwards 1956) The results obtained were rather variable In three experiments the space constant λ was found to be 0 41 mm, 0 23 mm and 0 36 mm, and it was found that the addition of acetylcholine and prostigmine lowered the space constant λ on the average by 29 per cent Values of the time constant lay between 3 6 and 9 8 msec and were reduced by about 50 per cent by acetylcholine plus prostigmine Values were obtained for the constants R R_m and C_m by the use of certain assumptions C_m was found to be 30 μf/cm^2, which is strikingly high The reduction in the space and time constants after the application of acetylcholine leads to the conclusion that the membrane resistance fell (Those who consult the paper of Trautwein et al 1956 should note that the value of C_m given there as 3 μf/cm^2, is a misprint and the value actually found was 30 μf/cm^2)

A drop in membrane resistance during the action of acetylcholine also has been found in dog atria (Trautwein and Dudel 1958*a*), and the resistance drop has been attributed to an increase in membrane permeability to K^+ It also has been stated (Dudel and Trautwein 1955) that under some circumstances epinephrine causes a fall in the membrane resistance of dog atrial fibers All the experiments cited on dog atrium have determined relative changes and as yet no values are available for the normal passive electrical properties of fibers of mammalian atria

ELECTRICAL EXCITABILITY

Only brief mention of the electrical excitability of the atrium will be made, since this aspect of cardiac electrophysiology has recently been considered in detail (Brooks et al 1955) and since atrial muscle has not been extensively employed for studies of excitability using transmembrane stimulation of single fibers If surface electrodes are used for stimulation it is found the electrical excitability of the atrium is in general similar to that of ventricular

muscle from the same heart Thus, if the same electrodes are used for both determinations, the resting thresholds of atrium and ventricle are the same in a given heart, and the diastolic strength duration curves are quite superposable The greater density of nerve fibers in atrial muscle introduces some difficulty in measuring excitability if relatively strong stimuli are required Perhaps for this reason the measurement of thresholds at various times during the relative refractory period is more variable than in the ventricle On the whole, however, the strength interval curve of atrium is quite similar to that obtained for ventricle in that anodal super normality is seen during the relative refractory period and cathodal supernormality is seen at the end of the relative refractory period The effective refractory period which is defined in terms of the ability of the heart to develop a propagated action potential, can be estimated from experiments similar to those shown in Chap 8 As in ventricular muscle (Hoffman, Kao, and Suckling 1957), the effective refractory period ends when the membrane of the indi vidual fiber has repolarized to a value of 60 to 70 mv Stimuli applied earlier than this elicit local responses which may become propagated when adjacent tissue is sufficiently repolarized (Kao and Hoffman, 1958) latency between stimulus and propagated response under these conditions may be considerably prolonged

During repolarization of atrial muscle there is a sharply circum scribed period during which suprathreshold stimuli give rise to multiple responses flutter, or fibrillation The extent of this vulner able period is changed markedly by vagal stimulation (Hoffman, Siebens and Brooks 1952) and other factors which influence repolarization or the regenerative response of the membrane (see Brooks et al 1955) In general it can usually be assumed that the recovery of excitability parallels the repolarization of the fiber membrane (for exceptions see Schutz 1936 Brooks et al 1955)

4

THE VENTRICLE

The musculature of the ventricles is normally activated at many different sites from the terminal branches of the Purkinje system This more or less simultaneous activation results in almost synchronous and forceful contraction of the entire ventricular muscle mass When one studies the electrophysiology of ventricular muscle the results suggest that these fibers are indeed the most highly specialized and developed part of all the cardiac tissues The specialization is such that vigorous contraction will take place under a wide variety of conditions capable of producing marked abnormalities in the function of other parts of the heart Fibers in all parts of the ventricle are remarkably uniform with respect to the magnitude and configuration of the transmembrane action potential Conduction velocity is quite constant Pacemaker activity seemingly does not develop in ventricular fibers Marked *changes in resting length have little effect on electrical activity* high concentrations of the autonomic transmitters similarly are without marked effect

THE VENTRICULAR ACTION POTENTIAL

Configuration

The transmembrane action potential recorded from ventricular fibers is usually represented as showing a rapid phase of depolarization a plateau of long duration fairly rapid repolarization and a steady level of transmembrane potential between action potentials This description requires certain modifications The three major

types of action potentials typified by dog frog, and rat, are shown in Fig 4 1 In each of these species ventricular fibers rarely if ever show spontaneous depolarization during phase 4 The onset of activity in phase 0 therefore is abrupt However, two major species differences in the ventricular actions potential are to be found These consist of the presence or absence of phase 1 and the presence or absence of a well marked phase 2 The only vertebrate species which are known not to show a well marked phase 2 or plateau are the rat and mouse All others which have been studied do show a plateau, the principal difference among them concerns the appearance of phase 1

A well marked phase 1 is seen in the action potential of the ventricle of many mammals, e g dog and cat (Hoffman and Suckling, 1952, Trautwein and Zink, 1952), sheep and calf (Délèze, 1959) Records from human ventricles published by Bromberger Barnea (Bromberger-Barnea et al 1959) reveal a suggestion of phase 1 at normal temperatures (see also Woodbury et al, 1957) The myocardium of birds (Hoffman unpublished) shows a well marked phase 1, in fact the rapid repolarization during phase 1 carries the membrane potential down to −40 mv so that the plateau occurs at a level halfway between the level of full depolarization and full repolarization Many other species apparently lack phase 1 entirely so that the plateau occurs at the level of full depolarization This form of action potential has been found in guinea pig (Johnson 1956 Coraboeuf and Otsuka 1956) snapping turtle (*Chelydra serpentina* and *Pseudemys elegans*) (Weidmann 1956*a*) frog (Woodbury et al 1951 Ware et al 1957), ground squirrel (*Citellus citellus*) (Coraboeuf Kayser and Gargouil 1956) and dog shark (*Scylliorhinus canicula*) (Gargouil and Coraboeuf 1957) Frog ventricle shows a fairly well marked phase 1 at higher temperature (Woodbury et al 1951)

Variation within the Same Heart Little systematic evidence exists on the question of variation in the action potential of ventricular fibers from different parts of the heart Electrocardiographic theory often assumes for convenience in interpretation of the T wave, that the duration of action potentials of the endocardial fibers is less than that of fibers of the epicardial surface This difference is however, often supposed to result from a difference in temperature One study (Matsuda et al 1956) has in fact reported that the

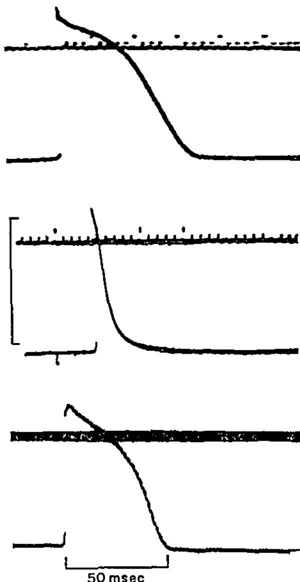


FIG 4-1 Transmembrane action potentials dog (top) rat (middle) frog (bottom) Time calibration for dog and rat heart shown by time marks at 10 and 50 msec on line of zero potential Time calibration on bottom trace is 500 msec *not* 50 msec Voltage calibration for all three records 100 mv

transmembrane potentials of fibers of the endocardial surface of the dog are shorter than those of the epicardial surface and show a more pronounced phase 1 Experiments in which a microelectrode was passed from the epicardial to the endocardial surface of the intact in situ dog ventricle (Hoffman and Suckling, unpublished observations) failed to reveal any consistent difference in the con figuration of action potentials of 20 to 30 cells penetrated at different depths A difference in duration of about 20 msec was noted This probably resulted from cooling of the exposed epicardial surface

Magnitude

The normal resting and action potentials of ventricular fibers are in all probability not less than 90 and 120 mv respectively The available evidence suggests two considerations in support of this statement One is that research conducted in our laboratory over a period of some years on the same type of cell has resulted in a steady increase in the average recorded values of both potentials This probably means that the earlier average values were low because of a lesser degree of technical proficiency Other direct evidence to sup port this statement is offered by studies of frog ventricle The early studies (Woodbury Hecht and Christopherson, 1951) reported rather low resting and action potentials The work has been repeated (Ware et al 1957) and much higher values have been found To obtain these it was necessary to use small electrodes and to take other technical precautions A table of values appears in Chap 3 all the values in that table may well be too low except those for Purkinje fibers which, because of their relatively large diameter and weak contraction are less likely than other cardiac fibers to be injured by the microelectrode Unusually low resting potentials (less than 80 mv) must be regarded as unproved at present The general tendency of recent experimentation is such as to place the burden of proof on the investigators who report low values The problem is discus ed again in Chap 7

Rate of Depolarization

The rate of depolarization during phase 0 of the action potential of ventricular fibers has been reported to range from 80 to 530 volts/sec The latter value was recorded from the endocardial sur

face of dog ventricle (Matsuda et al, 1956) and may represent the activity of cells transitional between myocardium and Purkinje fiber Low rising velocities are subject to the same criticism offered against low values of resting and action potential amplitude It seems reasonable to conjecture that the maximum rate of depolarization in mammalian myocardial fibers is at least 350 volts/sec That of frog and turtle is undoubtedly much lower, probably 50 volts/sec or less although measurements at temperatures which permit comparison with mammals are not available

Conduction Velocity

The conduction velocity in dog ventricle has been reported to be 0 88 m/sec (Schaefer and Trautwein 1949) and that in cat papillary muscle 0 96 m/sec (Trautwein Gottstein, and Dudel, 1954) These values are somewhat higher than the classically accepted 0 4 m/sec (Lewis 1925) but agree well with the authors observation of a conduction velocity of 0 9 to 1 0 m/sec in papillary muscles and trabeculae carnae of the dog heart Low values (0 3 m/sec) for conduction velocity in the intact in situ ventricle obtained from plunge electrodes (Scher et al, 1953 1955 Erickson et al 1957) should properly be interpreted in terms of what was actually measured, namely excitation times at a series of points The true distance between these points in terms of fiber length is unknown the values obtained by this method certainly do not represent conduction velocity Even as measurements of excitation time the values may be erroneously low because of local injury and block which is inevitable when any series of rigidly mounted electrodes is pushed through moving ventricular myocardium

Conduction velocity in frog ventricle is about 0 1 m/sec at 18°C (Bammer and Rothschuh 1952*a*, Bammer and Rothschuh 1952*b* Bammer 1953) As in Purkinje fibers conduction velocity in frog ventricle increases linearly with temperature At 3°C the velocity is 0 048 m/sec and at about 27°C it is 0 16 m/sec Above 27°C the rate of increase of velocity is slightly diminished (Heintzen, 1954)

Duration

The duration of the ventricular action potential in any one species is highly variable under different conditions There are also marked

differences in the action potential duration of hearts from different mammals In dog papillary muscle, for example, the action potential duration at a rate of 100 beats per minute is from 175 to 250 msec, in rat ventricle, at the same temperature and rate, the duration is from 50 to 70 msec In general the total duration of the action potential is difficult to determine because of the very gradual slope of the terminal part of phase 3 For this reason most investigators have preferred to measure action-potential duration to some point on phase 3 such as that representing 80 per cent restoration of the full resting potential A comparison of the action potential duration in hearts of different species is presented in Table 3 1 Changes in duration caused by a variety of factors and agents are discussed in subsequent sections of this chapter In general, deterioration due to any cause is associated with a decrease in action potential duration although a few exceptions have been noted (Webb and Hollander, 1959) In fibers which show a clearly marked phase 2, a decrease in the duration of this phase is most often responsible for shortening, only rarely is a decrease in action potential duration a result of an increase in the steepness of phase 3

THE VENTRICULAR ELECTROGRAM

A brief general description of the cardiac electrogram was given in Chap 1 If a unipolar electrogram is recorded directly from the surface of uninjured muscle through a small electrode the intrinsic deflection of the initial complex corresponds quite exactly in time to the upstroke of the local transmembrane action potential (Fig 4 2) Similarly, under normal conditions the *ST* segment corresponds to the plateau and the T wave to the phase of repolarization of muscle in the immediate vicinity of the surface electrode To a certain extent the unipolar electrogram can be employed to detect changes in the voltage time course of the transmembrane potential However, changes in the velocity of propagation as well as nonuniformity in the electrical activity of different fibers have some effect on the electrogram Ventricular muscle from dog heart provides a good example for discussion because of the clear temporal separation between depolarization and repolarization and thus between the R and T deflections For this reason several of the factors which have been shown to alter the ventricular transmembrane action potential will

be considered in relation to the unipolar ventricular electrogram of the dog

The only change recorded in the electrogram when preparations of ventricular muscle are driven at progressively higher rates is a

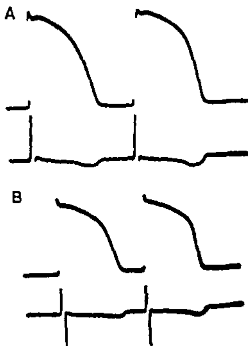


FIG 4-2 Transmembrane action potentials and unipolar electrograms recorded from isolated dog papillary muscle showing one driven beat followed by an early diastolic extrasystole (*A*) Normal Tyrode solution (*B*) low calcium solution Note changes in phases 2 and 3 of the transmembrane potentials and in the *ST* segment and T wave of the extrasystoles

change in the duration of the *ST* segment (Hoffman and Suckling 1954*a*) Since increa ed rate changes only the duration of phase 2 of the transmembrane action potential the absence of any modifica tion of the R and T deflections is not surprising Inversion of the T wave and other modifications which have been reported as a con sequence of increased frequency of contraction (Garb and Cheno weth 1953) result from hypoxia and inadequate perfusion If, on the other hand the increase in frequency is caused by some factor other

than the frequency of the driving stimulus, the ventricular electrogram may show other changes For example, during tachycardia due to epinephrine or norepinephrine there may be, in addition to a shortening of the *ST* segment, a decrease in the duration of the R wave and changes in amplitude and configuration of the T wave These changes result from the augmented conduction velocity and from the effect of sympathomimetic agents on the rate of change of transmembrane potential during phase 3

The relationship between the rate of repolarization during phase 2 and 3 and the voltage time course of the *ST* segment and T deflection have been studied in some detail If an extrasystole is introduced sometime during phase 4 the transmembrane action potential often shows a change in the slope of phases 2 and 3 (Fig 4-2*A*) The slope of phase 2 is decreased and that of phase 3 is increased The simultaneous unipolar electrogram shows no displacement of the *ST* segment from the isoelectric line and an increase in the amplitude and sharpness of the T wave A similar change in the transmembrane potential and in the electrogram is recorded when the extracellular Ca^{++} concentration is reduced (Fig 4-2*B*) These observations all suggest a consistent and direct relationship between the rate of change of the transmembrane potential during phases 2 and 3 and the magnitude of the *ST* displacement and T deflection Increased amplitude of the T wave, however does not necessarily indicate an increase in the slope of phase 3 This is clearly demonstrated in records obtained from ventricular muscle exposed to a moderate increase in the extracellular K^{+} concentration Under this condition the unipolar electrogram shows a marked increase in amplitude of the T wave although the simultaneous transmembrane action potential shows an *increase in the slope of phase 2* and a decrease in the slope of phase 3 The increased amplitude of the T deflection in these records therefore does not result from an increased slope in phase 3, actually it is caused in large part by the diminished conduction velocity This change in conduction velocity which is indicated by the increased duration of the R wave causes a greater asynchrony in the onset of repolarization in different fibers and thus an increase in the magnitude of the T wave The greater displacement of the *ST* segment arises in part from this same factor and in part from the increase in the slope of phase 2 of the transmembrane action potential

It has been observed that the electrocardiograms of mammals differ in that some show a clear *ST* segment and T wave while others show a T deflection merged with the terminal portion of the QRS complex (Lombard 1952) An example of the latter sort is found in the electrocardiogram of the rat in which the T wave is merged with the terminal part of the R wave This type of record is to be expected since the transmembrane action potential which is similar to that of dog and cat atrium, shows no clearly defined plateau (Fig 4-1) Of greater interest perhaps is the prolonged negative afterpotential recorded from single fibers of rat ventricle During this afterpotential the unipolar electrogram *fails* to show any deflection which might correspond to the U wave of the electrocardiogram (Surawicz et al, 1959) Similarly a U wave is not recorded from dog or cat papillary muscle even if the terminal phase of repolarization is prolonged Moreover recent studies of perfused rabbit heart (Hoffman Cranefield, Lepeschkin et al 1959) have failed to reveal any correlation between the appearance of a U wave in the electrogram and the presence or absence of afterpotentials in records obtained either through intracellular microelectrodes or small suction electrodes Discussion of other possible explanations for the U wave will be found in Chap 7

EFFECT OF PHYSIOLOGICAL VARIABLES

Rate and Rhythm

The influence of rate on the duration of the action potential of the ventricle has been the subject of interest for many years Many formulas have been proposed to relate the *QT* interval of the electrocardiogram to heart rate (see Lepeschkin 1951 Carmeliet 1955*a* and *b*) Equal interest has been shown by workers who have studied ventricular action potentials by transmembrane recording A brief summary of results obtained by those workers will be given, a more detailed consideration of the systematic studies on frog ventricle by Carmeliet will follow

Studies on dog papillary muscle (Hoffman and Suckling, 1954*a*) showed a linear relation between total duration of the action potential and rate when the rate was varied from 60 to 300 per minute The shortening resulted largely if not entirely from shortening of

phase 2, the slope of phase 3 remaining largely unaltered (Fig 4-3) At rates below 50 per minute the duration of phase 2 was constant These results are not necessarily comparable to the effect of heart rate on the *QT* interval of the electrocardiogram, since in the in

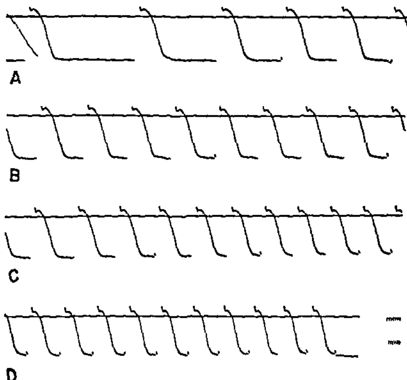


FIG 4-3 The effect of rate on the shape and duration of the transmembrane action potential of dog papillary muscle The initial rate is 73 per minute and the final rate is 273 per minute The time calibration in the lower right hand corner is a 60-cycle sine wave

situ heart rate changes may be caused by a variety of factors which influence action potential duration in other ways In the studies just described a small reduction of resting potential was noted only at very high rates No effect on conduction velocity was found unless the resting potential and thus the amplitude and rising velocity of the action potential, were decreased If a quiescent preparation was brought into activity at a fixed rate, the duration

of the action potential did not immediately stabilize The initial action potential was a little longer than the second one The third action potential was a little longer than the second but a little shorter than the first An alternation of this sort persisted for several beats The introduction of a single extrasystole in phase 4 produces an action potential with a prolonged phase 2 and a somewhat steepened phase 3 The paper contains a discussion of the relationship of these changes to changes in the T wave The influence of rate on action potential duration and mechanical activity in cat papillary muscle also has been examined (Trautwein and Dudel 1954a) The relationship between rate and duration in this report is somewhat complicated, however increase in rate leads to marked reduction of duration Some fall in action potential amplitude was noted at rates of 350 per minute and higher

A series of studies on frog ventricle by Carmeliet and his coworkers has provided a great deal of interesting information In the initial papers (Carmeliet 1955a and *b*) it is pointed out that the conventional formulas are not altogether adequate and it is proposed that action potential duration is influenced by some factor which disappears slowly after each beat If it is assumed that this factor declines in an exponential fashion, it is possible to obtain a simple formula relating the interval between beats T the duration at a given rate A the duration at infinitely slow rate A_∞ and the time constant of decline of the rate influencing factor, α The resulting formula which may of course also be regarded as empirical, is $A = A_\infty(1 - e^{- \ T})$ The values of A_∞ and α differ from heart to heart and as will be seen differ under differing physiological conditions The formula describes the observations of duration as a function of rate very well It should be noted that for slow rates (large values of T) A approaches A_∞ asymptotically This corresponds with the observation that no further lengthening of the action potential is seen when rate is reduced below a certain level

It will be seen that a change in physiological state may change either the duration seen at infinitely slow rate (A_∞) or the degree to which increase of rate leads to shortening (measured by α) Usually both change when the state of the tissue is changed If α is very large the duration will be insensitive to changes in rate so that reduction in α represents an increase in the sensitivity of duration to rate change

In a series of papers (Carmeliet and Lacquet, 1956, Carmeliet and Boulpaep, 1958, Carmeliet and Lacquet, 1958) the changes in A_∞ and α under the action of K^+, temperature, Na^+, and osmotic pressure are reported It is of particular interest to note that increase in temperature and in external K^+ concentration both shorten A_∞ but that increase in temperature diminishes the sensitivity of the fiber to rate shortening whereas increase of K^+ increases that sensitivity It was also found that A_∞ is more sensitive to a change in external K^+ in summer frogs than in winter frogs

The general conclusion (Carmeliet and Lacquet, 1958) is that α is a measure of the rapidity with which the ionic balance between the inside and outside of the fiber is restored after an action potential The studies of Carmeliet emphasize an important fact about the interaction of rate duration and any other physiological variable namely that the only way in which the rate effect can be allowed for in studies of the effect of other variables on duration is by determining *a rate-duration curve at each level of* the *other variable* Since changes in other variables change not only the duration of the action potential at low rate but also the sensitivity of duration to rate change there is no simple way to "rule out rate effects In particular driving at a fixed frequency can never rule out rate effects

As remarked above changes in rate do not in general affect resting potential, action potential amplitude or conduction velocity unless the rate is so great that each new action potential arises during the latter part of phase 3 of the preceding action potential One study of frog ventricle however has reported a very substantial fall in conduction velocity as a result of increased rate (Bammer, 1953)

Acetylcholine

As would be expected, the action potentials of mammalian ventricle are insensitive to the action of acetylcholine No effect on resting potential is found in cat or dog papillary muscle or in in situ dog ventricle (Hoffman and Suckling 1953) An enormous concentration (11×10^{-2} M) produced very slight increase in the slope of phase 2 and a corresponding decrease in the slope of phase 3 of dog ventricle (Hoffman and Suckling 1953) Other cholinergic agents have similarly little effect on dog ventricle On the other hand earlier studies with suction electrodes showed that 1 5 000 acetylcholine does speed repolarization in frog ventricle (Lueken and Schutz 1938) a similar

effect is seen in records of transmembrane potential from single fibers of frog ventricle (Hoffman unpublished) Acetylcholine has also been found to influence the effect of K^+ concentration on conduction velocity in frog ventricle (Bammer, 1952)

Epinephrine

Epinephrine and norepinephrine have very little effect on the amplitude of the resting potential or the action potential of dog or cat ventricular fibers according to studies in our laboratory It is particularly important to note that neither agent produces spontaneous depolarization during phase 4 Also epinephrine rarely if ever, causes spontaneous activity in preparations of dog ventricle which do not contain obvious Purkinje fibers This observation suggests that "ventricular" extrasystoles seen with the administration of these drugs actually result from spontaneous activity in peripheral twigs of the conducting system (see Chap 7) An effect of epinephrine on action potential duration of frog heart is noted in studies with the suction electrode, a considerable increase in duration follows the application of 1 5 000 epinephrine (Lueken and Schutz, 1938) In studies of isolated preparations of dog papillary muscle or trabeculae carneae (Hoffman unpublished) it has been shown that epinephrine causes a slight decrease in action potential duration at a constant heart rate by increasing the slope of phase 3 In the chick embryo on the other hand epinephrine increases the slope of phase 1 and decreases the slope of phase 3 (Fingl et al 1952)

The shortening of the QT interval associated with the action of epinephrine in the intact animal is largely a shortening secondary to a rate increase The change in configuration of the T wave results, in part, from changes in the slope of phase 3 and in part from the increased conduction velocity

Ions

Sodium Lowering of the external Na^+ concentration has one definite and reproducible effect on the action potential of ventricular fibers of most species namely it shortens duration Such a shortening has been seen in frog (Brady and Woodbury, 1957) snapping turtle (Cranefield Eyster and Gilson, 1951*a* Weidmann, personal communication) dog (Hoffman, unpublished) and sheep and calf (Délèze 1959) A fair amount of the shortening results from an

increased steepness of phase 2 while phase 3 occurs either at a normal rate or somewhat more slowly In general the slow repolarization during phase 2 proceeds further in terms of potential level, so that phase 3 begins only after the membrane is somewhat closer to full repolarization than when the extracellular Na^+ concentration is normal (Fig 4-4) The effect of very low external Na^+ on the appearance of the action potential is similar to the effect of acetylcholine

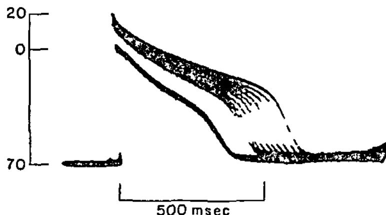


FIG 4-4 Successive action potentials of the same ventricular fiber Camera was opened during first 30 sec of perfusion with test solution containing 20 per cent sodium then again between 60 and 90 sec (*Délèze* 1959)

on atrial fibers or the effect of continuous anodal polarization on ventricular fibers In guinea pig heart however lowering of external Na^+ prolongs the duration of the action potential (Coraboeuf and Otsuka 1956)

The effect of reduced external Na^+ concentration on the steepness and amplitude of phase 0 is of course interesting because of its bearing on the Hodgkin theory It cannot be said that the results available to date give unequivocal support to the applicability of that theory to ventricular muscle In two species snapping turtle (Weidmann, personal communication) and guinea pig (Coraboeuf and Otsuka, 1956), it is possible to lower Na^+ markedly without apparent reduction in either the steepness or amplitude of phase 0 In a recent review (Cranefield and Hoffman 1958a) the results of Coraboeuf and Otsuka were queried because the NaCl was replaced by choline chloride The studies of guinea pig ventricle have been repeated

using both choline chloride and sucrose as NaCl substitutes, and the earlier findings were confirmed (Délèze, 1959) However it was noted that the upstroke velocity became reduced even though the amplitude of phase 0 remained normal In the study by Délèze it was found that replacement of NaCl by sucrose or choline chloride increased the force of contraction, and since such an increase is known to result from reduction of external Na^+ this finding was taken as evidence that the cells were exposed to low Na^+ Evidence that the guinea pig ventricle does not fully obey the Hodgkin theory is thus fairly strong It should be pointed out that several workers have studied the effect of low Na^+ on various types of cardiac fibers and have failed to publish their results even in abstract, because the results were negative i e, did not support the Hodgkin theory

Studies on frog ventricle (Brady and Woodbury 1957) have been interpreted as showing that that tissue obeys the predictions of the Hodgkin theory Thus the fibers become inexcitable if Na^+ is lowered to 15 per cent of normal, the amplitude of the action potential is reduced by reduction of Na^+ and the steepness of phase 0 is reduced by reduction of Na^+ However certain other aspects of this study are less easy to explain (Brady, personal communication) The decrease in action potential duration differs depending on the substituting substance, thus when choline chloride is substituted for NaCl a linear relation is seen between Na^+ concentration and duration such that a reduction of Na^+ by 1 mM reduces duration by 10 msec This shortening is transitory and after some minutes the duration begins to increase If sucrose is used as a substitute for NaCl shortening of the action potential is not seen with levels of Na^+ as low as 40 per cent of normal and in fact prolongation may occur The viscosity of a sucrose Ringer is high and it is possible that perfusion with this medium is ineffective in lowering extracellular Na^+ however this would not account for the prolongation observed In these particular studies two major assumptions must be made in order to relate the amplitude of phase 0 and the extracellular Na^+ concentration in a quantitative manner The amplitude of the action potential falls off in a predictable manner only as Na^+ is lowered to 60 per cent below 60 per cent amplitude decreases less than predicted This failure is explained on the supposition that intracellular Na^+ is lost (Brady and Woodbury 1957) Furthermore,

the fitting of a logarithmic relationship between Na^+/Na^+ and the amplitude of phase 0 even down to 60 per cent, depends upon the assumption that internal Na^+ concentration is considerably higher than it is usually supposed to be (Brady, personal communication) In addition the relationship between the steepness of phase 0 and Na^+ concentration is extremely variable and fails badly at low Na^+ concentrations All in all the results on frog ventricle cannot be regarded as giving strong support to the Na^+ theory, although it seems that the Na^+ concentration does bear some important relationship to the development of depolarization The technical difficulties in these experiments are great and results are difficult to interpret, so that it is not reasonable to reject absolutely the view that these experiments do in fact support the Hodgkin theory and that the departures are only the result of technical difficulties

The most recent results bearing on the effect of low Na^+ on ventricular fibers are those of Délèze (1959) Délèze used a preparation well adapted to the study namely the m transversus cordis This muscle is a bundle of myocardial fibers which in sheep and calf, *crosses the right ventricular cavity, connecting the interventricular* septum and the posterior wall and which carries an artery through which it can be perfused In these experiments NaCl was replaced either by sucrose or by a mixture of choline chloride and atropine No difference was found in the effects of these two substitutes A reduction of NaCl to 25 per cent decreased the action potential amplitude by 22 mv without changing the resting potential markedly The steepness of phase 0 was also reduced by 75 per cent The effect on action potential duration was striking, total duration being reduced by 50 per cent The reduction in amplitude of phase 0 is much less than the 37 mv predicted on the basis that the action potential amplitude is determined almost solely by the Na^+ potential It is suggested that the permeability to other ion species is therefore fairly high in comparison with the permeability to Na^+ Quantitative considerations indicate that if this explanation is correct, Na^+ permeability at the peak of the action potential is not much greater than the K^+ permeability Such an assumption raises serious problems *in relation to the supposed impedance changes* In spite of this the results of Délèze provide considerable support for the applicability of the Hodgkin theory to the ventricular muscle of sheep and calf hearts

In summary it may be said the effect of low Na^+ on ventricular muscle has been tested in four species In two of these species the results are not in accord with the Hodgkin theory In the other two species the results may be made to accord with the Hodgkin theory provided certain nontrivial assumptions are made The problem of the ionic basis of depolarization in cardiac muscle is a very important one and seems worthy of extensive investigation Above all it is important at present to maintain a distinction between experiments which may, with the aid of certain assumptions be interpreted in terms of the Hodgkin theory and experiments which give demonstrative evidence for the validity of the theory No unequivocal results of the latter kind have been reported as yet

Potassium The addition of KCl to a solution bathing ventricular muscle produces a lowering of the resting potential a reduction in the amplitude and upstroke velocity of phase 0 and a decreased duration These effects have been observed on guinea pig ventricle (Coraboeuf and Otsuka 1956) sheep and calf ventricle (Délèze 1959), turtle ventricle (Weidmann 1956a) frog ventricle (Brady and Woodbury, 1957) and dog ventricle (Brooks et al, 1955) Measurements of resting potential as a function of external K^+ concentration have been made (see Fig 3 12) and reasonable agreement with the Nernst equation was found in the authors unpublished studies Certain studies suggest that in frog (Brady and Woodbury, 1957) and turtle (Weidmann 1956a) the resting potential is not that which would be predicted by the Nernst equation An important difference between frog and mammalian ventricle has been revealed although not much consideration has been given to it In frog ventricle marked reduction of K^+ in the external medium greatly increases both the resting potential and the duration of the action potential Thus Carmeliet and Lacquet (1958) report that reduction of K^+ from 1 8 mM to zero causes a prolongation of some 20 per cent in action potential duration Similarly Brady and Woodbury (1957) found that reduction of K^+ to 25 per cent of normal caused a marked increase in resting potential amplitude of phase 0 and action potential duration These findings contrast with those made in studies of mammalian atrium (see Chap 3) mammalian Purkinje fibers (see Chap 7) and mammalian ventricle (Hoffman and Suckling 1956) In all those tissues reduction of K^+ to levels near zero causes a fall in transmembrane potential as well as other deteriorative changes

Action potential duration in frog ventricle has been found to vary linearly with the external K^+ concentration over a range from zero to 5.64 mM (Carmeliet and Lacquet, 1958). The relationship does not appear to remain linear with more marked increase in K^+, e.g., an increase in K^+ from twice normal to three times normal tremendously reduces duration (Brady and Woodbury, 1957). It has also been shown that an increase of K^+ increases conduction velocity in frog ventricle (Bammer and Rothschuh, 1952a, 1952b). This effect appears to be transitory if external K^+ is raised to very high levels, in which case the initial effect is an increase in conduction velocity and the persisting effect is depression of conduction velocity. Such observations accord with the concept that depolarization initially enhances excitability by bringing the resting potential closer to the threshold potential but eventually reduces excitability through inactivation.

Special attention must be paid to studies of the perfused turtle ventricle made by Wilde (Wilde et al., 1955, 1957). These studies used a method of "effluography," which depends upon perfusion through the coronary artery and collection of coronary venous outflow. If the ventricle is loaded with K^{42}, it is possible to collect samples of the effluent and determine their K^+ concentration. Use of this technique has shown that release of K^{42} from the turtle ventricle is pulsatile and that the pulsatility of the release has the same rate as the heart beat. Each contraction of the ventricle is associated with the appearance of an increase in K^+ in the effluent. Wilde (personal communication) estimates that 0.0026 of the K^+ content of the turtle ventricle is released during each contraction, or 1.3×10^{-5} meq K^+ per systole per gram of muscle. Rough calculations yield a value of 45 $\mu\mu$M K^+ per square centimeter per beat. Wilde also concludes that the K^+ release occurs during systole and that the K^+ release during systole is 8.3 times as great as the resting release of K^+. In analyzing Wilde's results Weidmann (1956b) has concluded that the change in transmembrane potential (and therefore in electrochemical gradient for K^+) during the action potential is adequate to explain the observed release of K^+. This conclusion may be taken to indicate that Wilde's findings do not demand a change in K^+ permeability during activity. On the other hand the calculations do not show that K^+ permeability does not increase. If the K^+ efflux occurs primarily during phase 3, then no doubt K^+ permeability does increase. The question remains unresolved.

The technique of perfusing turtle ventricle through its coronary system has been ingeniously applied to other problems by Weidmann (1956a). If the ventricle is cooled to 10°C, the action potential has a duration of 3 to 5 sec. Since substances injected into the coronary artery reach the vicinity of myocardial cells in about 1 sec, it is possible to alter the extracellular milieu during the course of the action

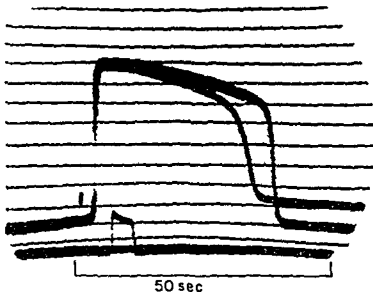


FIG 4-5 Transmembrane potentials recorded from perfused turtle ventricle showing one normal action potential and superimposed the shortening due to an injection of KCl into the coronary circulation. Time of injection shown by upward deflection of lower trace. Voltage calibration lines in steps of 10 mv (*Weidmann* 1957a)

potential. Thus it is possible to increase the extracellular K^+ concentration during phase 2 (Fig. 4-5). Such an increase leads to repolarization. This simple observation has raised many problems. If it is supposed that the value of the transmembrane potential during phase 2 is the result of roughly equal Na^+ and K^+ permeabilities, then an increase in the concentration of K_o^+ would, by reducing the magnitude of E_K, tend to move the transmembrane potential toward the Na^+ potential, i.e., in the direction of depolarization. The observed effect being quite the opposite of that predicted in the above manner suggests other interpretations, among them

1956a) or increase K^+ permeability (Cranefield and Hoffman, 1958a) The problem is discussed further in Chap 9

Calcium and Magnesium Comparatively few studies of the effect of Ca^{++} concentration have been conducted on ventricular fibers It has been found (Hoffman and Suckling, 1956) that great reduction in external Ca^{++} (to 1 per cent of normal) is without any remarkable effect on the transmembrane potentials of dog papillary muscle There is a very minor increase in resting potential no spontaneous depolarization develops during phase 4 and the steepness and amplitude of phase 0 are little altered Duration does increase by perhaps 15 per cent and phase 1 is slightly accentuated, so that phase 2 begins at a different level of transmembrane potential A fourfold increase in Ca^{++} on the other hand results in near abolition of phase 2, so that the action potential resembles the "atrial type" of potential Total depletion of Ca^{++} and Mg^{++} (by Sequestrene) results in great prolongation of phase 2 and a total duration of as much as 1 000 msec In spite of this prolongation there is little change in resting potential, phase 0 or phase 3 The level of Ca^{++} determines the effect of K^+ on the resting potential in exactly the same way it does in Purkinje fibers (see Chap 7) Changes in Mg^{++} are essentially ineffective unless Ca^{++} is 10 per cent of normal, under this condition the addition of Mg^{++} shortens phase 2, and the removal of Mg^{++} prolongs phase 2

Studies of the effect of Ca^{++} concentration on the monophasic action potential of frog ventricle (Rodeck, 1947) showed that, as Ca^{++} was elevated above normal minor elevation caused increased duration whereas higher concentrations either immediately or eventually caused shortening Shortening was seen only at concentrations higher than those employed by Hoffman and Suckling (5 to 25 times greater than normal) Rodeck s paper contains a summary and bibliography of early studies made with external leads A variety of contradictory results obtained on frog ventricle have been briefly summarized elsewhere (Cranefield and Hoffman 1958a)

Other Ions Cadmium chloride (5 mg/kg) has been found to lower the resting potential of frog ventricular fibers and also to increase the steepness of phase 2 thereby reducing duration (Kleinfeld Greene et al 1955) Barium chloride (5 mg/kg) markedly reduces the steepness of phase 3 and thereby prolongs the duration of the action potential in frog ventricle (Kleinfeld et al , 1954)

The effects of lithium chloride should be investigated further, since there is reason to believe that Li^+ can substitute for Na^+ in the production of phase 0 It has been found that 55 meq/l of LiCl can be added to a normal Ringer's solution without changing the transmembrane action potential of frog ventricle, on the other hand the replacement of 27 to 40 meq of NaCl by LiCl results in loss of amplitude in phase 0 and great reduction of duration (Stein et al 1955) The effect resembles a "low sodium' effect but replacement of a higher per cent of Na^+ by glucose does not produce nearly as marked effects Similar effects have been seen on snapping turtle ventricle (Cranefield Eyster and Gilson unpublished) It is suggested by Stein that normal Na^+ levels protect against the Li^+ effect On the other hand LiCl generally contains as an impurity the trivalent ions of various rare earths which were shown by Mines to be very toxic (Mines, 1910)

Temperature

The most obvious effect of cooling on the ventricular action potential is marked increase of duration without marked change in resting potential (Hoffman 1956) The change in duration results particularly from a decreased slope of phase 2 and from the consequent increase in the duration of phase 2 If however the tissue is cooled sufficiently resting potential is decreased and excitability is diminished or abolished (Woodbury et al 1951 Trautwein and Dudel 1954*b*, Gargouil and Coraboeuf 1957) It should be noted that the only study of the effects of temperature on the in situ dog ventricle (West personal communication) has not demonstrated any appreciable change in resting potential within the range from 37 to 27 C A similar study of human ventricle during hypothermia also failed to reveal a decrease in the magnitude of the resting potential or action potential at temperatures as low as 28 5°C (Bromberger Barnea et al , 1959) These latter findings are in part a result of the narrow range of temperatures employed but may also result in part from the greater experimental variation in magnitude of transmem brane potential measurements when flexibly mounted electrodes are used to study intact hearts

Many attempts have been made to formulate a quantitative description of the effect of temperature on the shape of the action potential Thus the temperature coefficients of the various phases

have been determined, and it is found that phase 2 is more temperature sensitive than phases 1 and 3 are. There is little basis for pressing such measurements very far and even less basis for elaboration of the data into physical or chemical hypotheses. On the one hand, as will be seen below, the description in terms of phases does not bear up well when examined critically. On the other hand it is difficult to reach meaningful physical chemical conclusions from a knowledge of the effect of temperature on a process as complex as that which eventuates in depolarization or repolarization (Burton, 1936–1937).

The description of temperature effects in terms of the effect on the maximum slopes of phases 1, 2, and 3 is useful. Were it to be precise, *it would imply that the action potential recorded at one temperature* could be superimposed upon that recorded at another merely by replotting the voltage time course with different time scales for each phase. Any examination of the action potentials recorded at low temperature shows that this is not so (Fig. 4-6). A rather detailed examination of this sort was carried out on frog ventricle by Heintzen (1954). He rejected the analysis based upon slopes of the various phases and instead chose to analyze the effect of temperature upon the time required for the action potential to change 10 per cent of its total amplitude. In other words he divided the phase of repolarization into 10 equal voltage steps and determined the duration of each as a function of temperature (Fig. 4-7). This method, also arbitrary, nevertheless produced very interesting results. The first three steps of repolarization (roughly phase 2) are quite sensitive to temperature and show a constant Q_{10} between 4 and 32.5°C; each, however, shows a different Q_{10} (Fig. 4-7). The phase of more rapid repolarization (Heintzen's steps 4 through 9, roughly phase 3) is quite insensitive to temperature down to about 15°C. Below 15°C these steps show marked prolongation and in fact prolong more rapidly than steps 1, 2, and 3. The last 10 per cent of repolarization shows a constant but low Q_{10}. These results may be regarded in two ways. They show that moderate reduction of temperature acts most markedly on phase 2, whereas more severe reduction of temperature produces a rather dramatic lengthening of phase 3. They also show that a change of temperature essentially changes the *shape* of the action potential in a way that cannot be represented by any simple analysis and in particular cannot be represented by the Q_{10} of phases 1, 2, and 3.

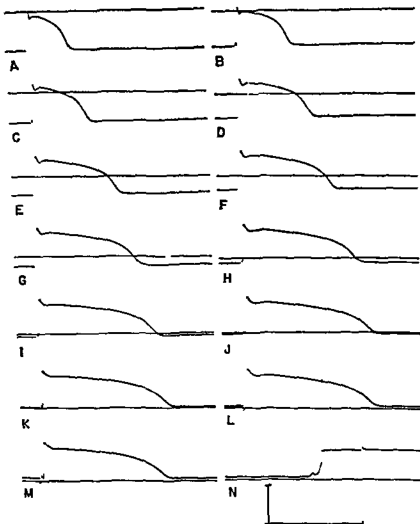


FIG 4-6 Transmembrane action potentials recorded from a single fiber of isolated cat papillary muscle at different temperatures Rate constant throughout at 30 per minute Top trace (in *A*) records muscle temperature which falls from 39 (*A*) to 19 C (*M*) Electrode withdrawn (*N*) to show line of zero potential Calibration represents 100 mv and 500 msec

Heintzen (1954) may also be consulted for a study of the effect of temperature on total duration, rate, and conduction velocity Interestingly complex seasonal variations in the effect of temperature on rate have also been reported (Smith, 1951, 1952)

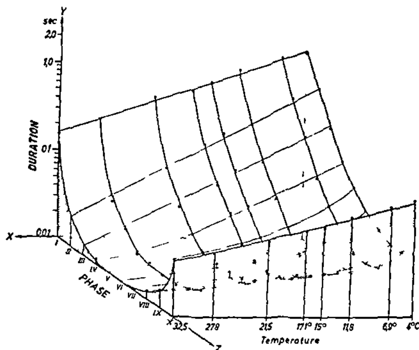


FIG 4-7 Three-dimensional graph showing the duration of the various phases of the action potential of frog ventricle as a function of temperature Each phase corresponds to a voltage change of 10 per cent of the total action potential amplitude Duration is plotted on a logarithmic scale on the *y* axis It will be noted that phases IV to VII change less in duration at high temperatures than do phases I to III but prolong more at low temperatures Only phases I II III and X show a constant Q_{10} over the entire range of temperatures (*Heintzen* 1954)

Drugs

It is not easy to demonstrate clear-cut effects of drugs on the ventricular action potential unless concentrations in excess of those used clinically are employed This should not be surprising if one remembers that action potentials are in general quite resistant to the effect of minor changes On the other hand caution must be

exercised in drawing conclusions from experiments in which drugs are used in high concentrations

Cardiac Glycosides A few studies on the effect of strophanthin on Ca^{++} deficient papillary muscles have been reported (Trautwein and Witt 1952, Dudel and Trautwein, 1958a) A concentration of 2.5×10^{-7} has little effect on amplitude but does produce a transitory prolongation of the action potential Subsequently duration decreases principally through a shortening of phase 2 A number of substances (digitoxin ouabain scillaren B, lanatoside C and K-strophanthoside) in high concentration produce similar effects on frog ventricle (Woodbury and Hecht, 1952) The most marked effect is a gradual disappearance of phases 2 and 3 so that repolarization is very rapid At clearly toxic levels the amplitude of phase 0 is reduced without reduction of the resting potential Similar results have been obtained on embryonic chick heart (Fingl et al, 1952) and on dog ventricular strips (Stutz et al 1954) Studies in the authors laboratory have failed to reveal any significant change in the transmembrane potentials of dog papillary muscle with therapeutic concentrations of ouabain

Quinidine Very low levels of quinidine (and procaine amide) prolong the effective refractory period of dog papillary muscle fibers without producing any other changes in the action potential (Hoffman 1958) the significance of this finding is discussed in Chap 7 Higher and probably toxic concentrations of quinidine sulfate (3 to 4 μg/ml) procaine amide (100 μg/ml) and pyrilamine maleate (2 5 μg/ml) produce reduction of the steepness of phase 0 in guinea-pig ventricle (Johnson, 1956) These observations emphasize the fact that the prolongation of the effective refractory period is a sufficient explanation of the therapeutic effect of quinidine

Metabolic Inhibitors A number of agents (iodoacetate dinitrophenol azide) have been found to produce little change in the resting potential of frog ventricle (Kleinfeld Stein et al 1955, Kleinfeld et al 1956 MacFarlane 1956) Iodoacetate causes increased steepness of phase 2 in frog ventricle (Kleinfeld Stein, et al, 1955) Studies on turtle ventricle utilizing external recording (Marshall 1955) show that iodoacetic acid reduces the refractory period and the QT interval Dinitrophenol on the other hand increases the refractory period while decreasing the QT interval These results suggest that inhibitors may alter the usual relationship between

repolarization and excitability and strongly suggest that studies of excitability should be part of any study of the effect of inhibitors

MacFarlane (1960) has studied the effect of certain enzymatic inhibitors on the duration of the action potential of frog ventricle Various agents such as sodium azide, dinitrophenol sodium cyanide and sodium iodoacetate, if used in sufficiently high concentrations, eventually result in considerable reduction of duration Two of these agents, dinitrophenol and sodium iodoacetate, initially produce lengthening In all cases spontaneous activity eventually appears When maximal shortening has developed, the duration is not further reduced by an increase in rate MacFarlane also examined the effect of external K^+ concentration on duration and compared that effect with changes produced by metabolic inhibitors He advances certain distinctions between the action of the inhibitors and of elevation of external K^+ The inhibitors act more slowly than does an increase in K^+, and the shortening induced by inhibitors is greater in proportion to the reduction of amplitude than is the shortening induced by K^+ However in this study it was not possible to make accurate determinations of the resting potential If one subjects the results to the criteria for the evaluation of the effects of inhibitors advanced by Shanes (1958, p 107) there seems little reason to accept the conclusion drawn from these results that "an active ion transport modulated by K_o^+ may be important in maintaining the plateau " An alternative suggestion made in the study seems more probable namely that metabolic inhibitors increase the permeability of the membrane to K^+ A nonspecific increase in K^+ permeability and a loss of any tendency for K^+ permeability to increase during a particular part of the cycle would certainly explain the observation that all the inhibitors produce a condition in which duration becomes insensitive to rate A very sharp distinction should be drawn between a plateau which depends upon metabolically sustained active ion transport during phase 2 and one which depends upon metabolically sustained permeability properties of the membrane MacFarlane also reports some interesting results obtained with neomonacrin, proflavine and acridine orange, all of which produce a slight lengthening followed by a slight shortening but which create a very marked prolongation after they are washed out with normal Ringer's solution The cells remain stained after

such washing out, and the duration may increase by nearly 100 per cent

Other Agents

Hypoxia Studies utilizing metabolic inhibitors seem to be more popular than studies of the effect of oxygen lack In general, however the effects of low-oxygen tension are similar to the effects of metabolic inhibitors The effects of hypoxia generally consist of increased steepness of phase 2 resulting in shortening of duration, slow loss of resting potential and gradual fall in amplitude (Trautwein Gottstein and Dudel 1954) The changes appear much more rapidly when the tissue is active than when quiescent muscle is exposed to low concentrations of O_2 (Coraboeuf, Gargouil Laplaud and Desplaces 1958) All knowledge available at present suggests that the various types of interference with metabolic activity including direct hypoxia produce effects remarkably similar to those produced by the elevation of K^+ in the external milieu A considerable burden of proof lies upon the investigators who advance mechanisms other than the one suggested by this fact

In a recent study of intact rabbit and dog hearts perfused through the coronary arteries (Kardesch et al 1958) it was found that cessation of the perfusion which resulted in anoxia as well, produced changes in the transmembrane potentials similar in magnitude and time of appearance to those caused by hypoxia alone (Trautwein and Dudel 1956 Webb and Hollander, 1956b)

Stretch Cat papillary muscles withstand stretch up to tensions of 1 000 g/cm² without showing altered transmembrane action potentials (Dudel and Trautwein 1954) It is unlikely that tension in the intact in situ heart often reaches this level

Fibrillation Various studies of fibrillation (Hoffman, unpublished Hoffman and Suckling 1954b Sano, Tsuchihashi and Shimamoto 1958) agree that single fibers of fibrillating ventricles may show reasonably normal action potentials succeeding one another at a very high rate All mixtures between this picture and one of small premature action potentials or action potentials with low upstroke velocity and reduced amplitude have been reported Also some investigators have observed a progressive decrease in the amplitude of the resting potential and the action potential and slowing of the

upstroke during prolonged fibrillation (Sano et al , 1958, Trautwein and Zink, 1952)

In intact, isolated cat hearts perfused from another cat (Hoffman and Suckling 1954b) the electrical activity of single fibers during prolonged ventricular fibrillation differs from the normal pattern only with respect to the high rate and a predominance of multiple premature action potentials These premature potentials naturally show changes in amplitude, upstroke velocity, and duration in proportion to the level of membrane potential preceding the upstroke If fibrillation is induced by an increase in the extracellular concentration of KCl, on the other hand the resting potential upstroke velocity and amplitude of the action potential are reduced to the same extent as during action of the same concentration of KCl during regular beats In fibrillation induced by KCl a given cell fires more slowly than it does either during spontaneous fibrillation or during fibrillation induced by electrical stimulation Only *in the presence of inadequate perfusion and hypoxia is there an* appreciable decrease in resting potential and action potential amplitude unless KCl is used to induce the arrhythmia These findings accord with the observation that in the unperfused heart fibrillation changes progressively and there is a decrease in amplitude and frequency of electrical activity recorded by surface electrodes, while in a heart maintained on a pump oxygenator, fibrillation may persist almost indefinitely without irreversible deterioration It is apparent therefore that changes of the type described by Sano and by Trautwein result from underperfusion with concomitant hypoxia and hyperkalemia and that, if perfusion is maintained, fibrillation of itself does not cause deteriorative changes in the electrical activity of the myocardium

One additional observation made during fibrillation may be of some importance As has been known for many years it is impossible to induce fibrillation in small pieces of ventricular muscle (Garrey 1914) Similarly it has not been possible to produce fibrillatory activity in isolated papillary muscles even though they are attached to bundles of Purkinje fibers in which multifocal pacemaker activity is observed (Stuckey, Hoffman, and Cranefield unpublished observations) Finally, even during ventricular fibrillation we have not observed pacemaker activity in ventricular muscle fibers

PASSIVE ELECTRICAL PROPERTIES

Very little is known about the cable characteristics of ventricular fibers. One early study (Kahn, 1941–1942) made on frog ventricle with external polarization and recording is still of interest, although it failed to determine the cable constants in a quantitative manner. Weidmann (1956b) has made certain estimates based upon the characteristics of Purkinje fibers and the chronaxie of ventricular fibers. These estimates point to a time constant of 10 msec and to a value of specific resistivity of the myoplasm similar to that found in Purkinje fibers, of about 153 ohm cm.

Some information which bears upon the impedance change during activity has been obtained by the use of impedance bridge measurements through external electrodes (Rosenblueth and del Pozo, 1943; Eyster and Gilson, 1947; Cranefield, Eyster and Gilson, 1951b). These measurements found an increase in impedance associated with activity. This finding is presumably similar to the finding that R_m is high during phase 2 of the Purkinje fiber action potential. Only one microelectrode study of changes in the membrane resistance of ventricular fibers has been reported. In this study (Coraboeuf, Zacouto, Gargouil, and Laplaud, 1958) it was found that the transmembrane resistance of fibers of the guinea pig ventricle falls during phase 0 and rises slowly during phase 2 to a level which is considerably less than during phase 4. The authors draw no conclusions about the value during phase 3, but it seems probable that the membrane resistance falls again during phase 3 before rising to the level characteristic of phase 4.

5

THE SINOATRIAL NODE[1]

Since early antiquity the spontaneous rhythm of the heart has been an object of curiosity and has often been identified as the ultimate essence of life itself (Kisch 1959) Despite this our knowledge of the nature of that rhythmicity has accumulated very slowly, and the sinoatrial node was not identified as the pacemaker of the mammalian heart until the second decade of this century The fact that in vertebrate hearts rhythmicity is a property of the cardiac muscle fibers themselves had become generally accepted by 1910, and attention turned toward the problem of localization of the pacemaker The results of that period, which are mentioned briefly below are discussed at length by Eyster and Meek (1921) and Lewis (1925)

Gaskell (1900) had found by a series of classical experiments that the normal pacemaker of amphibian hearts is in tissue of the sinus venosus The exact location of that pacemaker was later shown by Meek and Eyster (1915–1916) to be at the junction of sinus with atrium The description of the sinoatrial node of mammalian hearts by Keith and Flack (1906–1907) and others prompted many workers to seek the pacemaker of the mammalian heart in the region of the junction of the superior vena cava with the atrium Many techniques were successfully employed in an attempt to provide a clear demonstration of the fact that the sinoatrial node is the normal pacemaker of the mammalian heart The strongest evidence was obtained by electrical recording from surface electrodes (Wybauw 1910, Lewis et al, 1910–1911, Eyster and Meek, 1914, Meek and Eyster,

[1] The unpublished studies by B F Hoffman which are reported in this chapter were supported in part by a grant from the New York Heart Association

1914*a* *b*) This method clearly showed that the first part of the mammalian heart to depolarize during normal excitation is the tissue in the vicinity of the sinoatrial node This finding of initial negativity of the nodal area, in conjunction with the results of experiments involving the effects of local injury cooling heating or stimulation definitely established the site of origin of activity It should be noted that the initial negativity in many early records in all likelihood was the result of depolarization of atrial muscle in the immediate vicinity of the sinoatrial node The low rate of depolarization of pacemaker fibers and their low conduction velocity combine to produce only small differences of potential on the surface of the atrium which are very difficult to record through external electrodes

After the discovery of initial negativity in the region of the sinus node during normal impulse initiation shifts in the pacemaker site were studied by recording the local electrogram Meek and Eyster (1914*b*) and Eccles and Hoff (1934) showed that the site of primary negativity migrated to different parts of the node under certain conditions such as vagal stimulation cooling or local application of KCl Meek and Eyster (1914*b*) summarized their conclusions as follows

> On the basis of work presented in this paper and others of the series a theory has been presented which correlates our experimental results on the automatic and vagal mechanisms of the vertebrate heart It is believed that the specialized tissues of the heart exhibit from above downward progressively diminishing degrees of automaticity Vagal chronotropic innervation of the specialized tissue also diminishes from above downwards The most automatic portion of the specialized tissue acts as pacemaker for the heart The function of the chronotropic fibers is to depress this automaticity When the automaticity of the pacemaker is reduced below that of a lower part the latter assumes dominance and becomes pacemaker In this way the vagus if the stimuli are properly graded may cause the pacemaker to descend from the upper part of the sinus node where it resides normally to the lower part of the sinus node and finally even to the auriculoventricular node

The considerable accuracy of these conclusions can be judged in terms of the records of transmembrane potentials of single fibers described below Studies of this type failed, however, to demonstrate

the mechanisms responsible for a shift in pacemaker site or for a change in rate and rhythm

The nature of pacemaker activity in locations other than the sinoatrial node has also been the subject of much experimentation and hypothesis It was early proposed by some investigators that impulse initiation only occurs in specialized tissues (Schlomovitz, Eyster, and Meek 1915) However, the early demonstration that many different areas of myocardium from amphibia and mammals beat spontaneously under certain conditions seems to have resulted in the probably erroneous conclusion that all types of myocardial fibers can develop intrinsic rhythmicity This question is discussed below in the section on ectopic pacemakers Studies of pacemaker activity in the sinoatrial and atrioventricular nodes also led to a search for a specialized conduction path between the two nodal regions similar to that which connects the atrioventricular node and the ventricular myocardium The problem of the existence and functional significance of such a path is discussed in Chap 3

The method of external recording was also used in attempts to determine the nature of pacemaker activity The electrical activity of the normal pacemaker was variously described as a slow negative variation in potential (Arvanitaki and Cardot, 1937, Arvanitaki 1938, Bozler 1942–1943) or an oscillatory potential (Bozler, 1943) and was thought by some workers to arise in tissue of the superior vena cava rather than in the sinoatrial node (Rijlant 1932) Extremely refined surface recording techniques have recently been employed to study this same problem (van der Kooi et al 1956) Records obtained through small close bipolar electrodes show low voltage, polyphasic complexes which precede propagated activity in atrial muscle and which are seen only in the immediate vicinity of the sinoatrial node The use of an intracellular microelectrode to study pacemaker activity of amphibian and mammalian hearts is described in detail below

TRANSMEMBRANE POTENTIALS OF THE SINOATRIAL NODE

The Nodal Action Potential

Since the sinoatrial node is the normal pacemaker of the mammalian heart, most of the descriptive material relating to the

transmembrane potentials of pacemakers has been derived from experiments on this particular tissue Pacemaker activity in the atrioventricular node and the specialized conducting system is described in Chaps 6 and 7 There are a variety of sites in the right

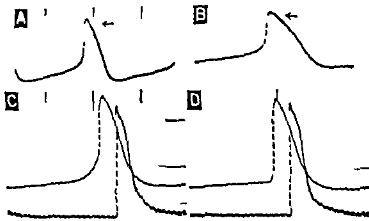


Fig 5-1 Transmembrane action potentials recorded from rabbit heart A and B Single pacemaker fibers of the sinoatrial node recorded at slow (A) and fast (B) sweep velocity Note gradual transition from slow depolarization of phase 4 to the more rapid depolarization of phase 0 Horizontal calibration lines represent 10 mv potential difference Line of zero potential indicated by arrows C and D Simultaneous records from a single atrial fiber (lower trace) and a true pacemaker fiber (upper trace) in *C* and a latent pacemaker fiber in *D* Voltage calibration shows 10 mv potential difference time calibration shows intervals of 40 msec

atrium which exhibit latent pacemaker activity Occasional reference to these latent pacemakers is made in this section, but they are described in detail in a following section

The fundamental difference between the transmembrane potentials of a pacemaker fiber and a fiber of either atrial or ventricular muscle is that the pacemaker fail to maintain a steady level of resting potential during phase 4 instead a slow depolarization commences immediately after the end of phase 3 (Fig 5 1) When the transmembrane potential reaches the threshold potential the record shows a smooth transition to the upstroke of the locally arising action potential This type of record was first obtained from pacemakers of isolated Purkinje fibers (Draper and Weidmann

1951) and single fibers of the frog sinus (Trautwein and Zink, 1952) *More recently (West, 1955a, West et al, 1956) similar records have* been obtained from single fibers of the mammalian sinoatrial node

In addition to showing slow depolarization during phase 4, the transmembrane potential of a single nodal fiber reveals several other characteristics The magnitude of the resting potential recorded from such fibers is less than that of atrial muscle, values of 50 to 65 mv are typical of the node in contrast to a resting potential of 80 to 90 mv in the atrium of the same species The action potential recorded from a single nodal fiber typically shows an extremely slow rising phase and the reversal is small or absent completely (Fig 5 1) The peak of the action potential is quite rounded, and repolarization is somewhat slow In a typical record only phases 2 and 3 seem to be present and phase 2 is often slow enough to be described as a plateau The records shown in Fig 5 1 are from nodal fibers of rabbit heart In other species (dog, cat, rat) the transmembrane potentials *from nodal pacemakers are similar to those of the rabbit* When records are obtained from different fibers in the same sinoatrial node the shape and magnitude of the action potential is found to vary considerably from fiber to fiber even within a small area In general, records obtained from fibers located closer to atrial muscle reveal a higher resting potential and a more rapid upstroke during phase 0 (West, 1955a) Fibers located in tissue of the superior vena cava at some distance from its junction with atrium, on the other hand, often have resting potentials as low as 30 to 40 mv and may show only graded responses to depolarization of the pacemaker In all nodal fibers, however, slow depolarization is seen during phase 4

Mechanisms Responsible for Rate Changes

Propagated activity arises when the spontaneous depolarization seen in phase 4 carries the membrane potential to the level of the *threshold potential* The interval between beats originating from a pacemaker cell thus reflects the length of time required for the spontaneous depolarization to carry the membrane potential from the resting potential to the threshold potential It is clear that this interval depends both upon the rapidity of the spontaneous depolarization and upon the amount of depolarization required to bring the fiber to the threshold potential A variation in swiftness of the spontaneous depolarization is found to be a very important mechanism

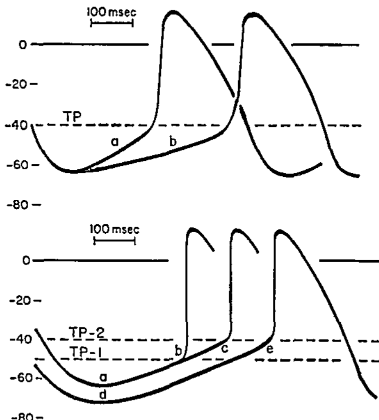


FIG 5-2 Diagram repre enting the important mechanisms responsible for changes in frequency of a pacemaker fiber The upper diagram shows a decrease in rate caused by a decrease in the slope of phase 4 from *a* to *b* and thus an increase in the time required for the transmembrane potential to reach the threshold potential (TP) The lower diagram shows rate changes associated with a change in the level of the threshold potential from TP 1 to TP 2 and an increase in cycle length from *a-b* to *a-c* also shown is a change in rate due to an increase in resting potential (compare *a-c* and *d e*)

in many changes of rate (Fig 5 2), it is seen during the action of acetylcholine of epinephrine and of temperature Rate may however, be varied even if the rapidity of the spontaneous depolarization is unaltered This occurs if the amount of depolarization required to bring the fiber to the threshold potential is changed, such a change results if either the resting potential or the threshold potential is

altered (Fig 5-2) There are thus at least three factors which are important in determining the frequency of discharge of a pacemaker namely, the steepness of the spontaneous depolarization, the level of the resting potential, and the level of the threshold potential It will be seen below that rate changes in the intact heart may come about in a somewhat different fashion in that the original pacemaker may be slowed so much that another fiber takes over the initiation of activity

Changes in both rate and rhythm are seen when one or more ectopic pacemakers fire in addition to the regular pacemaker Under unusual circumstances such as the presence of toxic concentrations of epinephrine or veratrine, it appears that certain fibers may remain partly depolarized and thus initiate repetitive firing Finally, certain changes in rate and rhythm which often appear as coupled beats, may result from echoes occurring in some part of the specialized conducting system The mechanism responsible for this phenomenon has not yet been studied in a satisfactory manner and the local changes in transmembrane potential responsible for this arrhythmia are uncertain Recent studies however have provided strong evidence that persistent local depolarization at a junction between fibers with dissimilar properties is one mechanism by which reentrant activity can be produced (Hoffman, Kao and Suckling 1957, Hoffman, Cranefield and Stuckey, unpublished)

The Vagus and Acetylcholine The first demonstration of the action of acetylcholine on pacemaker activity came from studies of the frog sinus venosus during vagal stimulation (Castillo and Katz 1955, Hutter and Trautwein, 1955*a* *b*) In this tissue vagal activity slows the heart rate primarily by decreasing the slope of slow depolarization during phase 4 With stronger vagal activity arrest of the pacemaker results from a hyperpolarization of the pacemaker fibers, which may amount to 20 to 30 mv The effect of vagal stimulation is greatest during phase 3 and least marked just prior to the upstroke of the action potential Also the hyperpolarization resulting from a fixed intensity of vagal activity is more marked when the resting potential of the pacemaker fiber is low In these same experiments conducted at 16°C it was shown that the duration of the vagal effect after a single stimulus was approximately 5 to 7 sec In the frog sinus venosus the level of the threshold potential is unchanged during vagal stimulation and action potentials arising from a pace-

maker slowed by vagal activity are decreased both in amplitude and duration The change in duration is much less than that seen in adjacent atrial fibers (Hutter and Trautwein 1956)

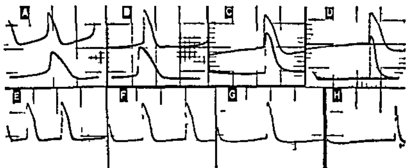


Fig 5-3 Records showing the effects of acetylcholine on pacemaker fibers in different parts of the rabbit atrium demonstrating the variability of the effect of this agent (*A*) and (*B*) Simultaneous records from the same pacemaker fiber in the sinoatrial node recorded at fast (lower trace) and slow (upper trace) sweep velocities showing in the control record (*A*) marked depolarization during phase 4 and a gradual transition from phase 4 to phase 0 and after addition of a low concentration of acetylcholine (*B*) a slight increase in resting potential a decrease in the slope of phase 4 and an abrupt transition from phase 4 to phase 0 as propagated activity reaches the recording site (*C*) and (*D*) Simultaneous records from a latent pacemaker fiber and an atrial muscle fiber in another heart (*C*) Control record (*D*) effects of a moderate concentration of acetylcholine The two superimposed sweeps in *D* show that slow depolarization during phase 4 is not abolished instead the transmembrane potential falls to a steady level before the upstroke of the propagated action potential (*E*) (*F*) (*G*) and (*H*) Records from a latent pacemaker in the same atrium as *A* and *B* showing (*E*) control records (*F*) a decreased slope of phase 4 in a low concentration of acetylcholine and (*G* and *H*) some hyperpolarization as well as a decrease in the amplitude and duration of the action potential in the presence of a higher concentration of acetylcholine Note that the transmembrane potential tends to attain a steady level prior to the onset of propagated activity

Vagal effects on the sinoatrial node of the mammalian heart have not yet been recorded The actions of acetylcholine are however similar in many respects to the effects of the vagus on the frog sinus West (1955*a* West et al 1956) reported a decrease in the slope of phase 4 an increase in resting potential and a decrease in the amplitude of the action potential after local application of acetylcholine to the sinoatrial node of the rabbit (Fig 5 3*A* and *B*) Change in the threshold potential was difficult to evaluate because of shifts in the

pacemaker site Other studies of the same preparation (Hoffman, unpublished) confirmed most of these results, but an increase in the maximum value of the resting potential was observed only infrequently More often the addition of acetylcholine to the perfusion fluid produced only a minor change in slope of the initial part of phase 4 of the pacemaker fiber The transmembrane potential fell to a level somewhat above the threshold potential and then remained constant until propagated activity from another pacemaker caused firing of the original pacemaker fiber (Fig 5 3*C* and *D*) In the presence of higher concentrations of acetylcholine complete arrest was associated with a steady level of membrane potential intermediate between the resting and threshold potentials Similar results have been obtained by Trautwein and Dudel (1958*b*) In the sinoatrial node of the rabbit acetylcholine causes no appreciable change in the duration of the action potential The magnitude of the sinoatrial pacemaker action potential however, is often reduced The threshold potential of pacemaker fibers is not increased by acetylcholine, a slight decrease cannot be ruled out because of the shift in pacemaker site mentioned earlier The effect of acetylcholine on other atrial pacemakers in the mammalian heart appears to be similar to that described for fibers of the sinoatrial node In general it seems that higher concentrations of acetylcholine are required to change the slope of phase 4 in latent pacemakers than in nodal fibers The action potential of some latent pacemaker fibers like that of a sinoatrial node fiber is little shortened by acetylcholine This finding is in contrast to the marked shortening caused by acetylcholine *in ordinary atrial fibers and fibers of the sinoatrial ring bundle* (Fig 5 3*E* through *H*)

The effect of acetylcholine on pacemaker activity is conditioned to some extent by other factors When the atrium is cooled (see below), acetylcholine fails to cause arrest of sinoatrial pacemakers Similarly, if the extracellular potassium concentration is elevated above the normal range higher concentrations of acetylcholine are required to produce slowing or arrest than in the presence of a normal potassium level Other interrelationships between potassium and acetylcholine are discussed in subsequent sections of this chapter Several studies have shown that the effects of acetylcholine on rabbit heart are imitated qualitatively by physostigmine and anticholinesterases and are blocked by atropine in high concentrations

Detailed comparisons of the effects of the parasympathomimetic agents and parasympathetic blocking agents have not been reported

A number of studies have given some insight into the mode of action of acetylcholine on fibers of the sinoatrial node It has been shown that the potassium permeability of atrial muscle is increased by acetylcholine Holland et al (1952*a* *b*) and more recently Harris and Hutter (1956) have demonstrated an increase in both the influx and efflux of potassium from guinea pig atrium and from frog and tortoise sinus venosus during acetylcholine action The membrane resistance of fibers from frog atrium (Trautwein, Kuffler, and Edwards 1956) and dog atrium (Trautwein and Dudel 1958*a*) is decreased by acetylcholine These observations taken in conjunction with the decreased effectiveness of acetylcholine in the presence of an elevated extracellular potassium concentration strongly suggest that vagal slowing is due to an increase in potassium permeability of nodal fibers The increase in permeability decreases the loss of membrane potential during phase 4 and results in slowing or arrest The significance of these observations in terms of the mechanism of slow depolarization during phase 4 is discussed below

Sympathetic Nerves and Sympathomimetic Amines The effects of the cardiac sympathetics on transmembrane potentials of pacemakers have been studied only in the frog heart (Hutter and Trautwein 1956) Stimulation of the sympathetics in an atropinized preparation causes an increase in the slope of phase 4 and also an increase in the slope of phase 0 and in the amplitude of the overshoot The threshold potential is seemingly unchanged although most published records have shown some shift in the location of the pacemaker An increase in the resting potential of quiescent preparations of frog sinus has been observed following stimulation of the sympathetics When epinephrine is added to the sinoatrial node of the rabbit heart (West, 1955*a* West Falk, and Cervoni, 1956) or the cat or rat heart (Hoffman unpublished) the most marked effect is an increase in the rate of depolarization during phase 4 However in the sinoatrial node of rabbit and cat hearts epinephrine seems to lower the threshold potential of true pacemakers (Hoffman unpublished) The effect of norepinephrine appears to be the same as that of epinephrine Several other sympathomimetic amines similarly increase the intrinsic rate by increasing the slope of depolarization during phase 4 (Morris Hoffman Kelly and Cranefield unpub

lished) When epinephrine is used in high concentration or is applied locally (West, 1955a) the effects on fibers of the sinoatrial node are various In addition to an increase in the slope of phase 4 and a frequent shift in the pacemaker site there may appear local block or slurring and notching of the upstroke of the nodal action potential Similar effects are produced in latent pacemakers surrounding the sinoatrial node

Unfortunately, studies of the membrane resistance of fibers in the sinoatrial node under normal conditions and under the influence of epinephrine have not been reported The observation (Dudel and Trautwein 1955) that epinephrine increases the resting potential and lowers the membrane resistance of dog atrium is difficult to relate to its mode of action on pacemaker fibers especially in view of the effects of acetylcholine described above and the observation that epinephrine increases permeability of the membrane to potassium (Hajdu, 1953)

Temperature Marshall (1957) has studied the effects of cooling on rabbit sinoatrial node and has observed the same decrease in the slope of phase 4 with cooling which is seen in Purkinje fiber pacemakers In addition, she noted that there was less change in the resting potential of pacemaker fibers during cooling than in adjacent atrial muscle At 10 to 13°C, when the atrial muscle had become inexcitable as the result of a low resting potential electrical activity of the pacemaker was maintained Under these conditions addition of acetylcholine raised the resting potential of atrial muscle and restored propagation without producing a marked change in the frequency of the sinoatrial pacemaker This effect of acetylcholine is seen in a number of conditions in which the excitability of atrial muscle is reduced because of a low resting potential The effects of acetylcholine on rabbit atrium described by Bülbring and Burn (1949) are in all likelihood to be explained in this manner

Spontaneous activity in sinoatrial pacemakers of the rabbit heart ceases at temperatures below 10°C because the slow depolarization during phase 4 fails to attain the threshold potential (Marshall 1957) As the temperature is increased to 38 to 39°C, the slope of phase 4 increases progressively, at temperatures in excess of 43 to 44°C, there is a decrease in the amplitude of both the resting potential and the action potential In addition, multifocal pacemakers appear, and conduction block within the node is frequent

Ions The effects of the major ions on pacemaker activity in the sinoatrial node have been studied only to a limited extent In rabbit heart the slope of phase 4 is relatively insensitive to alterations in the concentration of Ca^{++} (Fig 5-4), but a marked increase in the extracellular concentration of this ion has an effect somewhat similar

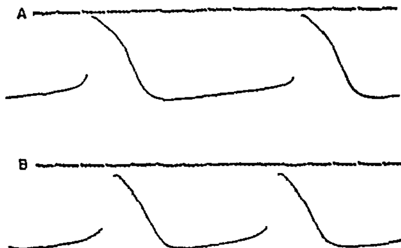


FIG 5-4 Transmembrane potentials recorded from pacemaker fibers of rabbit atrium in (*A*) normal Tyrode solution and (*B*) a solution containing one-half the normal concentration of Ca^{++} (*Unpublished records Walmor Carlos d Mello*)

to that of acetylcholine Complete Ca^{++} depletion may increase the slope of phase 4 and lower both the resting potential and the action potential These studies have not been carried out in the presence of atropine, and it is thus uncertain to what extent acetylcholine participates in producing the observed changes A change in the Na^{+} concentration of the perfusion fluid has relatively little effect on the slope of phase 4 in rabbit sinoatrial node When 50 per cent of the NaCl is replaced by an isosmotic amount of sucrose the slope of phase 4 is unchanged or slightly diminished, replacement of 75 per cent of the NaCl causes a general deterioration, but spontaneous depolarization persists and sometimes is actually increased If most of the Cl^{-} of the perfusion fluid is replaced by either NO_3^{-} or acetate pacemaker activity persists and the slope of phase 4 is more

or less unchanged for periods of 30 to 45 min or longer (Hoffma unpublished)

The sinoatrial node of the rabbit heart appears to be remarkab insensitive to changes in the extracellular K^+ level (Fig 5 5) T

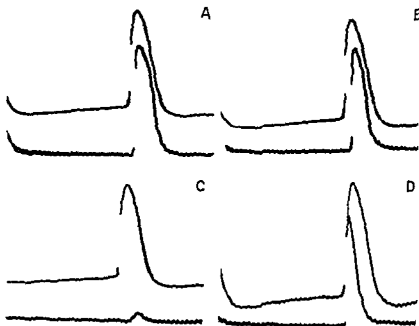


FIG 5-5 Simultaneous records from single fibers of a latent pacemaker fibe *(top trace) and muscle fiber (bottom trace) of isolated rabbit atrium (A)* Con trol K^+ concentration 3 mM/l *(B)* K^+ concentration increased to 10 mM/l *(C) 25 min in high* K^+ *solution (D) 25 min in* K^+ *free solution Note in C tha* activity persists in the pacemaker fiber at a time when the high K^+ solutio *has blocked activity in the atria fiber See text for discussion*

slope of phase 4 is usually not increased unless the potassium con centration is reduced below 0 5 mM/l In K^+ free solution spon taneous depolarization is increased and multifocal pacemaker activ ity appears When the concentration of K^+ is increased, the effect on sinoatrial pacemakers is not comparable to the effect on adjacent atrial fibers In the rabbit heart (de Mello unpublished, Hoffman, 1959) an increase in the extracellular K^+ concentration from the normal value of 2 7 to 10 0 mM/l causes little alteration either in the magnitude of the resting potential of sinoatrial pacemakers or in the slope of depolarization during phase 4 This same concen

tration of K^+ produces a marked drop in the resting potential of atrial muscle At an extracellular K^+ concentration of 13 5 mM/l pacemaker fibers continue to show regular, spontaneous action potentials propagation of this activity into the muscle fibers of the atrium is decremental, and at a distance of several millimeters from the sinoatrial node the record of transmembrane potential of atrial fibers shows only small nonpropagating depolarizations The resting potential of the nodal fibers is decreased only slightly, as is the slope of slow depolarization during phase 4 In atrial muscle at such K^+ concentrations, depolarization is marked (Fig 5 5 Fig 3 12) However when the K^+ level is increased to 21 or 22 mM/l spontaneous activity of pacemakers ceases, and an appreciable decrease in resting potential is evident

The apparent insensitivity of fibers of the sinoatrial node to K^+ has important implications The persistence of normal pacemaker activity at a time when the K^+ induced depolarization has caused a failure of propagation in atrial muscle provides a clear example of sinoatrial block It is possible that this mechanism is sometimes responsible for the disappearance of the P wave in the electrocardiogram during K^+ intoxication in most instances however the failure to record a P wave in surface leads results from a marked decrease in the propagation velocity in the atrium and from changes in the amplitude and rising velocity of the atrial action potential (Cranefield and Hoffman unpublished observations) Direct leads from the atrium under conditions of K^+ intoxication similar to those observed clinically show a persistence of propagated electrical activity in atrial muscle and a complete absence of a P wave in the surface electrocardiogram The lack of sensitivity of the sinoatrial node to changes in K^+ concentration may also explain the persistence of electrical activity in pacemaker fibers when atrial tissue has been made inexcitable by cold digitalis and other factors known to influence K^+ permeability and transport

Other Factors The sinoatrial node is quite sensitive to changes in fiber length Moderate stretch produces a decrease in resting potential an increased slope of phase 4 action potentials of reduced amplitude and multifocal pacemaker activity (Hoffman unpublished) A decreased partial pressure of O_2 or increased partial pressure of CO_2 have similar effects to those described for Purkinje fiber pacemakers although information on the relative sensitivities of the two

types of pacemakers is not available (Hoffman, unpublished). One study of the effect of metabolic inhibitors on the electrical activity of single fibers of the sinoatrial node of the rabbit heart (de Mello, 1959) has demonstrated a decrease in the slope of depolarization during phase 4 when dinitrophenol or azide were added to the perfusion medium. The effect of DNP was reversed by appropriate concentrations of ATP. Digitalis, even in high concentrations, does not abolish slow depolarization during phase 4 (West, personal communication). Furthermore, quinidine and procaine amide, in concentrations known to affect Purkinje fiber pacemakers, do not cause an appreciable change in the slope of phase 4 in normal pacemakers of the sinoatrial node (Hoffman, unpublished).

Other Properties of Nodal Fibers

Conduction Velocity. Measurements of conduction velocity within the sinoatrial node are difficult to make even with the use of intracellular microelectrodes, because the spread of activity is difficult to trace. It has been determined, however, that conduction time from the sinoatrial nodal pacemaker to the crista terminalis of the rabbit heart is approximately 35 to 45 msec. Assuming a linear spread of activity at a uniform rate, the conduction velocity over this distance is from 0.1 to 0.2 m/sec (Fig. 5.7). Actually, velocity increases with distance from the pacemaker, and the calculated value is erroneously high. It is likely that in the immediate vicinity of the pacemaker activity spreads at a rate of 0.05 m/sec or less (Paes de Carvalho et al., 1959). Since the rate of propagation is so slow, it would not be surprising if many sinoatrial nodal fibers reached the threshold potential almost simultaneously. Records of transmembrane potentials obtained at numerous locations within the node support this concept. A number of fibers usually can be found which appear to fire spontaneously, and thus normal pacemaker activity may be in this sense multifocal in origin. The conduction velocity in the sinoatrial node is so slow that the first fiber to reach threshold may not be the pacemaker at all. Propagation from such a fiber may not reach atrial fibers until they have already been activated by an impulse originating in a nodal fiber which is not the earliest fiber to reach threshold but which is closer to the atrial fibers.

Excitability. In most cardiac fibers (see Chap. 8) full recovery of excitability seems to follow almost immediately after the completion

of repolarization The level of the transmembrane potential is related to excitability and responsiveness in a manner similar to that described for Purkinje fibers (Weidmann, 1955a) In single fibers of the sinoatrial node this relationship often fails during the phase of

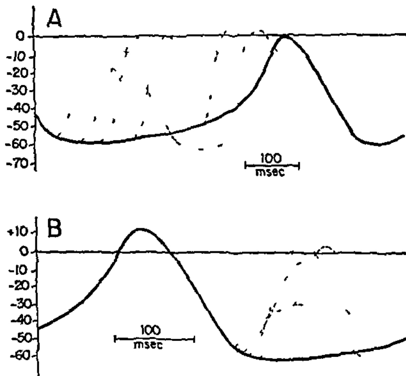


FIG 5-6 Tracings of records of transmembrane action potentials recorded from a single fiber in the sinoatrial node of the rabbit heart showing the responses to stimulation at various times during the phase of slow diastolic depolarization (*A*) and during the latter part of repolarization of a different fiber (*B*) See text for discussion

slow repolarization Thus, when a nodal fiber is stimulated at various intervals after the end of phase 3 and during phase 4, the amplitude and rising velocity of the response may increase as the level of trans membrane potential falls (see Fig 5-6) Studies of the effect of rate on action potentials of the sinoatrial node have shown that the maxi mum effect on duration is far less than that seen in atrial muscle

ervoni, West, and Falk, 1956) On the other hand, it also has been own that the amplitude of the nodal action potential varies rectly with the threshold potential (Hutter and Trautwein, 1956) ese two observations are most readily understood if we assume at in fibers of the sinoatrial node refractoriness outlasts repolariza n In terms of the ionic hypothesis this might mean that the neral relationship between transmembrane potential and activity intains but that the time constant of reactivation is much larger an in ordinary atrial or ventricular muscle

Safety Factor Several types of observation suggest that the safety ctor of the nodal action potential is quite low In many instances tivity originating in the atrium shows decremental conduction d local block in the sinoatrial node Similarly pacemaker activity a particular nodal fiber may fail to excite the remainder of this ucture or may fail to propagate to the atrium Under certain treme conditions the entire sinoatrial node may show one rhythm d the atrium another Specific examples of sinoatrial block due to y temperature, high extracellular potassium concentrations, and her factors are mentioned elsewhere in this chapter

Surface Electrograms from the Sinoatrial Node As mentioned in the roduction to this chapter a variety of potentials have been corded from the region of the sinoatrial node through surface elec des Since we have no information on the passive electrical prop ies of nodal fibers it is difficult to predict the shape of the surface ctrograms from a knowledge of the time course of the transmem ane action potential However certain generalizations are proba permissible Slow depolarization during phase 4 is present in a ge number of cells distributed over a fairly wide area Because of slowness of this change in potential there is little difference ween the surface potential at different locations in the node, and se bipolar electrodes will show little or no evidence of this phase electrical activity A unipolar electrode and high gain d-c ampli will record the slow depolarization quite faithfully, although the gnitude of the signal will be small A somewhat similar situation encountered after the onset of propagated depolarization The ng phase of the nodal action potential is so slow that only very all bipolar electrodes will show an appreciable difference of poten l and then only if the electrode separation is relatively large uring propagation the slow conduction velocity and *irregular*

spread of activity will most often result in low voltage, polypha ic deflections in the bipolar electrogram without sharp, clear inflections Again, unless the time constant of the amplifier is long much of the activity in the nodal fibers will fail to be recorded, and the initial deflection of the record will represent the more rapid activity of latent pacemaker fibers which surround the sinoatrial node proper

OTHER ATRIAL PACEMAKERS

Latent Pacemakers

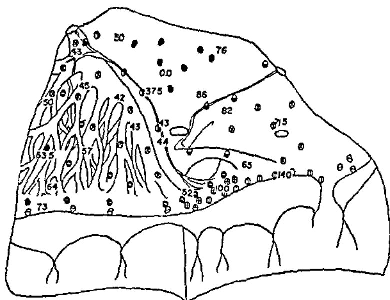
Surrounding the sinoatrial nodal tissue, which in rabbit hearts is located in the wall of the superior vena cava there is a rather wide region in which slow depolarization occurs during phase 4 Trans membrane potentials of single fibers in this region are intermediate in appearance between those of sinoatrial node and atrial muscle (West 1955*a*) The resting potential is somewhat higher than in the sinoatrial node, and the slope of depolarization during phase 4 is less Most important, the slow depolarization abruptly changes into the upstroke of the action potential at a level of transmembrane potential higher than the threshold potential (Fig 5 1) Under nor mal conditions these fibers are latent pacemakers they take over true pacemaker function when the sinoatrial node is depressed by acetylcholine or some other means

In addition to latent pacemakers lying between the sinoatrial node and the crista terminalis a number of other fiber groups in the atrium show slow depolarization during pha e 4 The distribution of these fibers is seen in Fig 5-7 In general they differ from atrial muscle not only in showing slow depolarization but also in the shape of the action potential and in their response to various agents (see Chap 3) These fibers which are thought to represent a form of muscle somewhat similar to the ventricular Purkinje system (ee Paes de Carvalho et al , 1959) are also latent pacemakers

Ectopic Pacemakers

Since many conditions and agents are known to initiate pacemaker activity in different parts of the atrium either on local application or after systemic administration a number of theories have been advanced to explain this phenomenon More particularly, it

has been assumed that any cardiac muscle fiber can become a pacemaker. Also, it has been postulated that the mechanism responsible for spontaneous firing in ectopic foci may be different from that in normal pacemakers (Scherf et al, 1953). The many studies of atrial



● SA pacemaker ◑ SA ring bundle ◒ septal branch of SA ring bundle; ⊛ musculi pectinati, crista terminalis and interatrial septum ◐ atrial roof, ⊖ AV ring, ⊗ AV node ⊕ His bundle

Fig. 5-7. Map showing spread of excitation in a preparation of the right atrium of rabbit heart. Figures represent latency in milliseconds with respect to the pacemaker located in the sinoatrial node (0 0). Type of action potential recorded from each site is indicated in the figure. Latent atrial pacemaker activity is found in the sinoatrial ring bundle, the crista terminalis and the atrioventricular ring in addition to the sinoatrial node. (*Paes de Carvalho et al 1959.*)

muscle carried out by means of intracellular microelectrodes permit certain tentative conclusions about the location of ectopic atrial pacemakers and the mechanism of their intrinsic rhythmicity.

Location. In studies of the right atrium from dogs, cats, rabbits, and rats, we have never recorded the development of pacemaker activity in ordinary atrial muscle fibers. Ectopic foci have always

been located in specialized fibers These fibers are found around the sinoatrial node in the region of the sinoatrial ring bundle (see Chap 3) in the specialized tissue of the atrioventricular ring and in the lower part of the atrioventricular node and bundle of His These fibers are the source of ectopic firing whether ectopic firing results from depression of a more rapid pacemaker or from increased automaticity of ectopic foci caused by physiological or pharmacological agents Little recent information is available on the presence or absence of latent pacemakers in tissue of the left atrium Erlanger (Erlanger and Blackman 1907 Erlanger 1910) stated that the left atrium isolated from the interatrial septum only rarely showed spontaneous rhythmical activity He also found (Erlanger 1910) that strips of the right atrium showed spontaneous activity only if they included one or more of those areas of atrial tissue which subsequently have been shown to contain specialized fibers (Paes de Carvalho et al 1959) This suggests that his method was valid and makes his failure to find spontaneous activity in the left atrium good evidence for a paucity of pacemaker tissues in that chamber If there are areas in the left atrium which do contain specialized pacemaker tissue, they are probably to be found posteriorly in the tissues surrounding the entry of the pulmonary veins and adjacent to the interatrial septum Isolated left atrial preparations ordinarily would not include this area

Mechanism The cause of spontaneous firing in ectopic atrial pacemakers is the same as that responsible for automaticity of the sinoatrial node Slow or rapid depolarization during phase 4 is thus the immediate cause of ectopic activity under most conditions However several apparent exceptions have been observed In some instances when the frequency of the ectopic focus is extremely high the upstroke of one action potential follows almost immediately after the end of the preceding phase 3 Even in this instance there is a smooth transition from the maximum resting potential at the end of phase 3 to onset of the succeeding phase 0 However in such a pacemaker the maximum resting potential is often almost identical to the threshold potential The cause of spontaneous firing in this case thus may be quite different from that responsible for normal rhythmicity An extreme example of this sort is produced in ventricular Purkinje fibers by veratrine (Hoffman and Matsuda, *unpublished*) or by toxic concentrations of epinephrine and norepinephrine and is dis-

cussed in Chap 7 Persistent local depolarization, which has been suggested as a possible cause of spontaneous activity, has not been observed in our studies of atrial activity Evidence that such a mechanism can initiate an action potential is provided by studies of anodal repolarization (see Chap 8), its operation in an ectopic atrial focus remains to be demonstrated

Another possible cause of ectopic activity should be mentioned, the phenomenon known by the term *echo* It is possible for propagated excitation to enter a region of cardiac muscle and exist there as an excitatory state until the end of the effective refractory period in adjacent fibers The persistent excitatory state (which is actually the normal long depolarization of phase 2) may then initiate a new propagated action potential in these particular fibers The conditions most favorable for establishment of this type of rhythm are seen in pacemaker fibers of the sinoatrial node when the excitability of this structure has been depressed If the normally low conduction velocity is further reduced and local blocks are present the following sequence of events may ensue Excitation initiated in one part of the node may cause normally propagated activity in the atrium This atrial activity will rapidly reach and excite some other area of the sinoatrial node Activity will then spread slowly over some pathway in the node and reexcite the atrial muscle shortly after the end of its effective refractory period A mechanism of this sort may cause either coupled beats a doubling of the normal rate or complete irregularity A conclusive demonstration of this sequence of events would require many simultaneous records of transmembrane potential at different locations and is not presently available However, a number of suggestive observations have been made In the first place even under normal conditions it appears that much of the nodal tissue is excited from the crista terminalis rather than from previously activated nodal fibers (Paes de Carvalho et al 1959) (see Fig 5 7) Secondly coupled rhythms in rabbit and cat atria have often been observed under conditions associated with depression of nodal activity and the occurrence of local blocks Finally, such coupled rhythms are most frequently associated with only a minor change in the sequence of activation of the atrium suggesting that the initial sites of excitation for both the normal and coupled beat are quite close to the sinoatrial node Observations made during studies of single fibers in the atrioventricular node also

lend support to this sequence of events as a possible mechanism for arrythmias (see Chap 6)

Although studies of the electrical activity of single pacemaker fibers have, from time to time revealed multifocal pacemakers and regional block this technique has made no particular contribution to our knowledge of the two most important atrial arrhythmias fibrillation and flutter Rapid regular rhythms similar to clinical flutter, have been observed in isolated atrial muscle preparations (Hoffman and Suckling 1953) This arrhythmia has been produced only in preparations of right atrium which contained some part of the crista terminalis and adjacent specialized tissues and only after addition of acetylcholine in a concentration sufficient to cause moderate or marked shortening of the atrial action potential Under these conditions the electrical activity of atrial muscle fibers is regular with respect both to direction of spread and amplitude of the transmembrane action potentials The cause of rapid activity is probably a single pacemaker firing in the usual manner at a greatly increased rate It is tempting to assume that the onset of spontaneous activity in more than one ectopic pacemaker, coupled with regional blocks may be the cause of atrial fibrillation Records of the activity of single atrial muscle fibers obtained during fibrillation (Hoffman and Suckling 1953) show marked variations in the amplitude of the transmembrane action potential similar to those observed during fibrillation of the ventricles (see Chap 4) and thus support the concept that local conduction block does occur Also, multifocal pacemaker activity has been produced in a variety of ways However, it has not yet been possible to obtain a clear experimental demonstration of the role of these two factors in the production or maintenance of fibrillation

The Sinus Venosus

Records obtained from the sinus venosus of frog and turtle hearts (Hutter and Trautwein 1956) are in large measure similar to those obtained from the mammalian sinoatrial node Slow depolarization during phase 4 is recorded from a large number of fibers in all parts of the sinus Close to the sinoatrial junction true pacemaker activity is seen The resting transmembrane potential of pacemaker fibers is lower than that of atrial muscle and the action potential shows only a small overshoot or no reversal at all The course of repolarization

is distinctly different from that of atrial fibers in that the clearly marked plateau of the latter is absent and phases 2 and 3 tend to merge quite smoothly In latent pacemaker fibers the resting and action potentials are intermediate in shape and amplitude between those recorded from atrium and from true pacemakers In general most of the statements made about fibers of the sinoatrial node also apply to pacemakers of the sinus venosus certain exceptions, however, are noted in other sections of this chapter

NORMAL REGULATION OF HEART RATE

There have been no reports of microelectrode studies of pacemaker activity in the sinoatrial node of intact in situ hearts, and thus any positive statement about the normal mechanisms by which rate is regulated is impossible However, on the basis of many studies of isolated preparations it is possible to bring forth reasonably convincing evidence for the hypothesis advanced many years ago by Meek and Eyster (1914*b*) which is quoted at the beginning of this chapter As a result of several types of investigation they concluded that vagal slowing was accomplished by a decrease in automaticity of one pacemaker area and the ensuing dominance of another pacemaker located either in a lower part of the sinus node or, with extreme slowing in the atrioventricular node Studies of most agents which change the automaticity of the mammalian sinoatrial pacemaker support this concept A change in rate is almost invariably associated with a shift in the pacemaker site In the case of vagal slowing this shift is from the sinoatrial node to adjacent latent pacemakers, from there to the specialized atrial fibers (see Chap 3), and finally to fibers in the bundle of His (see Chap 6) Since these effects have been produced by addition of acetylcholine to the perfusion medium, it would appear that the several types of pacemaker fibers differ in their sensitivity to this agent whether there is also a gradation in the intensity of vagal action has not been established Although recent studies have shown important local differences in the effect of vagal stimulation on excitability of atrial muscle (Alessi et al, 1958), it is not clear that this is not due to local differences in sensitivity to acetylcholine

Similarly, if the frequency of an isolated preparation is appreciably changed by addition of epinephrine or any other agent there is

usually a concomitant shift in the pacemaker site These observations strongly support the idea that major changes in rate result from suppression of one pacemaker and dominance of another rather than from change in the rate of the original pacemaker Minor adjustments in frequency, on the other hand, may be brought about by changes in the slope of phase 4 or in the magnitude of the threshold potential of the original pacemaker

THE CAUSE OF INTRINSIC RHYTHMICITY

General Considerations

Pacemaker fibers wherever found are characterized by slow depolarization during phase 4 Most attempts to interpret this phenomenon have been based on the Hodgkin theory In terms of that theory the slow depolarization represents a shift in the transmembrane potential from a level close to the K^+ equilibrium potential E_K toward the Na^+ equilibrium potential E_N Weidmann (1956*b*) suggested three possible causes for slow diastolic depolarization in Purkinje fibers (1) a decrease in the K^+ permeability, (2) a gradual increase in Na^+ permeability, and (3) a reduction in the activity of the Na^+ pump One should perhaps add to these possibilities a change in the permeability of the membrane to other ions Another theory which has been advanced (Cranefield and Hoffman, 1958*a*) depends upon the possibility that pacemaker cells contain more Na^+ and less K^+ than do nonpacemaker fibers In such a cell the level of E_K would be shifted in the direction of depolarization An increase in P_K associated with repolarization would bring the transmembrane potential close to E_K the subsequent decrease in P_K to the level characteristic of phase 4 would permit the ionic currents carried by Na^+ or other ions progressively to lower the membrane potential and thus provide the characteristic slow depolarization during phase 4 In this interpretation no special permeability changes would be necessary The same sequence of permeability changes postulated for the repolarization of ordinary cells would in a cell with the abnormal ionic concentrations described result in pacemaker activity Trautwein (Trautwein 1957 Trautwein and Dudel 1958*b* Dudel and Trautwein 1958*b*) has advanced a theory which is an elaboration of Weidmann s first possibility namely, that slow

depolarization results from a progressive decrease in P_K throughout phase 4. Before evaluating these several possible mechanisms it is necessary to summarize the pertinent experimental results.

Experimental Evidence

Studies of Membrane Impedance. Both in Purkinje fiber pacemakers (Weidmann, 1951) and in pacemaker fibers of the sinoatrial node (Dudel and Trautwein, 1958*b*) membrane impedance increases progressively during phase 4. It is not known whether this change in impedance is a decrease from or to normal because a quantitative comparison of the resting membrane impedance of nonpacemaker and pacemaker fibers is not available.

Effects of Extracellular Na^+ *Concentration.* Draper and Weidmann (1951) replaced part of the NaCl in Tyrode solution by sucrose and noted that the rate of depolarization of Purkinje fiber pacemakers was roughly proportional to the fraction of normal Na^+ in the *extracellular fluid.* A similar direct relationship has not been found in studies of sinoatrial pacemakers in the rabbit heart (Hoffman, 1959); in this tissue low Na^+ concentrations often resulted in an increased rate of depolarization. Dudel and Trautwein (1958*b*) also found that low Na^+ does not markedly reduce the slope of phase 4 in rabbit sinus. They found that lowering external Na^+ to 20 per cent of normal did reduce both resting potential and action potential amplitude.

Effects of Extracellular K^+ *Concentration.* The changes in pacemaker activity caused by alterations in the K^+ level in the extracellular fluid have been described in detail in another part of this chapter and in Chap. 6. The most important observation is that the rate of depolarization of Purkinje fibers during phase 4 is increased by low K^+ and decreased by high K_o^+ (Hoffman and Suckling, 1956). Pacemaker fibers of the sinoatrial node appear to be quite insensitive to marked changes in K_o^+ (de Mello, 1959) but show changes of similar direction.

Effects of Other Ions. Moderate changes in the extracellular Ca^{++} level do not change the slope of phase 4 of Purkinje fiber pacemakers (Weidmann, 1955*b*); more marked depletion increases the rate of depolarization of true and latent pacemakers in Purkinje fibers. Sinoatrial pacemakers are influenced less strongly by changes in Ca^{++} than are Purkinje fibers. Replacement of Cl^- in the perfusion

medium by either NO_3^- or acetate has little effect on depolarization of sinoatrial pacemakers during phase 4

Effects of Other Factors The rate of depolarization of all pacemakers during phase 4 is strongly affected by changes in temperature (Coraboeuf and Weidmann 1954 Marshall, 1957) in much the same manner as phase 2 Acetylcholine, which is known to increase potassium permeability, decreases the slope of phase 4 of sinoatrial pacemakers In frog and turtle hearts hyperpolarization is produced, in rabbit heart the membrane potential stabilizes at some level between the resting potential and threshold potential Epinephrine which also increases K^+ permeability (Hajdu, 1953), increases the slope of phase 4 in both sinoatrial and Purkinje fiber pacemakers

Ionic Content of Pacemakers The concentrations of Na^+ and K^+ in specialized tissues from ox Purkinje fibers and frog and turtle sinus venosus are compared to values for atrium and ventricle in Table 5-1 It is clear that in terms of these studies the pacemaker fibers contain much less K^+ and relatively more Na^+ than do fibers of atrial and ventricular muscle

TABLE 5-1 ELECTROLYTE CONTENT OF VARIOUS PARTS OF THE HEART

Species	Tissue	Cations mM/kg wet weight			Reference
		Na^+	K^+	Ca^{++}	
Frog	Sinus venosus	124	44 5	1 8	Mazel and Holland 1958
	Atria	87	42 2	1 8	
	Ventricle	49 7	68 2	2 0	
Turtle	Sinus venosus	129 4	56 8	3 5	Mazel and Holland 1958
	Atria	64 0	57 8	3 5	
	Ventricle	49 8	66 0	1 4	
Ox	AV bundle	159	93		Davies et al 1952
	AV node	155	61		
	SA node	153	57		
	Left atrium	72	86		
	Left ventricle	55	91		

Interpretations

The results of measurements of membrane impedance obviously suggest a progressive decrease in the ionic permeability of pace

maker fibers during phase 4 Ordinarily the increase in impedance is thought to indicate a decrease in K^+ permeability Dudel and Trautwein (1958b) have presented a theory based on this change which is similar in certain respects to that advanced by Shanes (1958) They consider the maximum transmembrane potential attained at the end of phase 4 to be an afterpotential due to high P_K, the subsequent slow depolarization during phase 4 represents the return of the transmembrane potential to the resting potential because of a decrease in P_K to a normal value Studies of the effects of changes in K_o^+ and Na^+ and of acetylcholine are in quantitative agreement with this hypothesis

It must be emphasized that impedance measurements of the type described are difficult to execute and extremely difficult to interpret When two microelectrodes are inserted near to one another in a syncytium of contractile fibers it is unlikely that the change in the amplitude of a square pulse applied through one electrode and recorded through another is due solely to changes in membrane resistance While these problems are reduced during diastole, it must be pointed out that many published records do not appear to represent changes solely caused by an IR drop through the membrane

A decrease in the slope of phase 4 when the extracellular Na^+ concentration is reduced merely indicates that some inward current is carried by this ion Similarly, the decreased rate of depolarization during phase 4 which is caused by acetylcholine would be expected regardless of the primary cause of slow depolarization In tissues in which acetylcholine causes hyperpolarization such as the frog sinus venosus it may be assumed that the membrane potential is less than the potassium equilibrium potential because of a low P_K, although other possibilities are obvious When acetylcholine fails to cause hyperpolarization as in rabbit sinoatrial node this interpretation is less convincing The effects of changes in K_o^+ are the reverse of the effects of this ion on phase 2 (see Chap 4) and suggest that, as in fibers of frog sartorius P_K in pacemaker fibers may be inversely proportional to the electrochemical driving force Low K_o^+ *would thus increase* the rate of depolarization during phase 4 because of a decrease in P_K (see Chap 9)

Several groups of investigators have suggested that the synthesis of acetylcholine induces automatic activity in cardiac muscle (Spadolini, 1953, Burn, 1956, Holland 1957) Recently it has been

shown that the sinus venosus of the frog heart differs from atrial and ventricular muscle in its higher content of acetylcholine cholinesterase, choline acetylase, and Na^+ and lower content of K^+ (Mazel and Holland, 1958) These findings have been interpreted as supporting the theory that intrinsic rhythmicity stems in some manner from acetylcholine metabolism Electrophysiological studies neither confirm nor refute this hypothesis It would be of interest to know whether or not *the development of automaticity in the embryonic* heart parallels the appearance of the various components of the acetylcholine system

Since it is not yet possible to describe a metabolic basis for the transmembrane potentials of nonpacemaker fibers, speculation as to the role of certain enzyme systems in the production of intrinsic rhythmicity is of little avail All that we can assume at this time is that depolarization during phase 4 is due to a change in net membrane current and thus to a change in permeability to one or more ions Many agents which alter rate act by causing an additional change in permeability or in available Na^+ carrier (Ca^{++} cocaine antifibrillatory agents see Chap 7) The marked effects of temperature and of so called metabolic inhibitors suggest that certain types of chemical reactions govern the time course and perhaps also the extent of the changes in permeability At present however, metabolic information can be related to the electrophysiological observations only by the use of broad assumptions which do not usefully substitute for experimental evidence

6

THE ATRIOVENTRICULAR NODE[1]

It has been known for some time that the delay between atrial contraction and ventricular contraction cannot be accounted for solely by the time required for an impulse to be conducted through the atrium and the Purkinje system Some definite delay occurs between the moment when the impulse reaches atrial fibers very near the bundle of His and the moment when electrical activity appears in the bundle of His It has become commonplace to attrib ute this delay to the passage of excitation through the anatomical area described by Tawara as the atrioventricular node This belief was challenged by Erlanger as early as 1912 and a great deal of recent work has tended to support his surmise that a large part of the delay occurs at the border between the atrial fibers proper and the atrioventricular node as defined anatomically The distinction may appear trivial particularly since a full correlation between recent electrical studies and modern anatomical studies has yet to be made but it seems necessary to remark that a major part of the *atrioventricular delay may not occur in the atrioventricular* node proper but rather at the junction of atrium with the node Our own preference at the present time is to use the term atrioventricular node in a loose sense to mean the entire complex of fibers function ally interposed between atrial fibers proper and His bundle fibers proper This loose usage is convenient because it makes it possible *to refer to the well known functional characteristics* of this area as

[1] The numerous unpublished results from our laboratory which are described in this chapter were obtained in the course of work supported by a grant from The National Heart Institute (USPH Grant H 3916)

characteristics of the atrioventricular node and to avoid the necessity of circumlocution The solution of choice would be to introduce a new anatomical term such as "atrioventricular junctional region" and to ignore the coincidence of the existence of a 'node' which happens to be part of the atrioventricular junctional region The continued use of the term atrioventricular node seems indicated at present New anatomical studies may present us with a new term, but for many years the term atrioventricular node has been nearly synonymous with "area in which the functional pecularities of the atrioventricular delay occur' and it seems reasonable to continue that usage temporarily

The functional peculiarities of the atrioventricular node are numerous and diverse and their consequences are often thought of more or less teleologically Transmission of excitation from atrium to ventricle is delayed during passage through this structure and the duration of this delay is adjusted to changes in heart rate by activity of the vagus and sympathetics Atrial impulses are transmitted through the node only up to a certain frequency, above this limiting value increasing degrees of block and complete failure of transmission develop The spread of excitation through the node results in simultaneous excitation of the fibers of the His bundle and thus permits almost synchronous activation of the mass of ventricular muscle In the absence of a more rapid pacemaker the atrioventricular node may take over the function of impulse initiation Finally it is asserted that conduction through some part of the node is slower in a retrograde than in a normal direction A large part of this chapter will be devoted to the description of recent experiments on single fibers of the atrioventricular node and specialized conduction system which have been conducted in an effort to explain some of these remarkable properties of this part of the cardiac syncytium In this introduction a brief survey of results of other types of studies is given

Studies Made with External Electrodes

Experimental Methods Many of the studies of the atrioventricular node have been indirect in nature In some the effective refractory period of the node has been estimated from measurements of the minimum time interval between atrial and ventricular beats during spontaneous or induced changes in rate and rhythm and during

normal and retrograde conduction These studies will be discussed in detail in the section on evidence for a dual atrioventricular conduction system It might be said at this time, however, that there is no reliable evidence in a study of this type to indicate the exact point of failure of transmission which may result in either delay or interruption of atrioventricular or retrograde conduction It has been demonstrated that delay and block can occur during retrograde conduction at the junction of ventricular muscle with the peripheral Purkinje fibers (see Chaps 7 and 8 Hoffman, Kao, and Suckling 1957) and that the duration of the action potential of nodal fibers is less than that of fibers in the His bundle Fibers of the His bundle in turn have a shorter action potential than do the peripheral Purkinje fibers Therefore the only conclusion permitted by these experiments is that the effective refractory period measured in this manner is the longest effective refractory period in a series of dissimilar fiber types

A number of attempts have been made to record the electrical activity of the atrioventricular node and His bundle by means of surface electrodes (Eyster and Meek 1916, van der Kooi et al, 1956 Scher et al, 1959) and also in some cases to stimulate nodal tissue through small surface electrodes Although several workers have succeeded in obtaining good records of the action potential of the His bundle and peripheral Purkinje fibers (Alanís et al, 1958, Scher, 1955) the action potential of nodal fibers (see below, Hoffman Paes de Carvalho de Mello and Cranefield 1959) is difficult to record even with relatively small surface electrodes Such records as have been obtained with monopolar recording techniques (Scher *et al* 1959) *present many difficulties in interpretation* Similarly experiments in which an attempt has been made to stimulate nodal fibers through surface electrodes have not shown that the stimuli were not in fact exciting either atrial or His bundle fibers The most fruitful experiments by far were those in which normal atrioventricular transmission was surgically interrupted at various points (Hering 1910, Eyster and Meek, 1916) and records made of the resulting sites of impulse initiation and subsequent delays in the transmission of excitation to atrium and ventricle

Evidence for the Nature of Nodal Transmission The most important experimental evidence bearing on the special properties of the atrioventricular node can be summarized briefly A normal atrioven

tricular delay is seen in electrocardiograms recorded from the embryo long before the appearance of a discrete atrioventricular node or bundle of specialized conducting fibers (Patten, 1956) Experiments on adult hearts which employed interruption of the atrioventricular conduction system at various levels combined with electrical stimulation above and below the point of block (Hering 1910, Erlanger, 1912 Eyster and Meek 1921) clearly demonstrated that the normal delay in atrioventricular transmission takes place at or in the atrial end of the node that the delay is localized to a fairly circumscribed area, and that after interruption of the normal atrioventricular pathway within the atrium the pacemaker site is usually located in the lower node or upper His bundle (Eyster and Meek 1916) Records of electrical activity obtained through small bipolar surface electrodes located at the upper end of the node have shown polyphasic low voltage deflections during the interval between activity in the atrium and His bundle (van der Kooi et al, 1956) Finally, these polyphasic deflections are remarkably similar to those obtained with the same electrodes from the immediate vicinity of the sinoatrial node prior to the onset of propagated activity in the atrium

Postulated Mechanisms of Delay A number of theories have been advanced to account for the normal atrioventricular nodal delay and for other special aspects of transmission through this structure It was for instance postulated that delay in conduction from atrium to ventricle results from the spread of activity at normal velocity over circuitous pathways or at reduced velocity over short paths composed of small diameter fibers Another possible mechanism was based on the assumption that some fibers in the path between atrium and ventricle have an unusual requirement for stimulus duration and are activated only during the repolarization of adjacent atrial fibers (Gilson 1942)

A study by Erlanger (1912) is of great importance, and a detailed consideration of his findings is in order At the time that he wrote the paper in question many physiologists regarded the atrioventricular interval as the result of slow conduction in the Purkinje system Erlanger therefore determined the conduction velocity in this system He did this in the ventricle of a beef heart under Langendorff perfusion He measured the interval between excitation of a false tendon and the subsequent contraction of the ventricle False tendons were cut and stimulated near their insertion into a papillary muscle

and at a distance from it The difference in the time elapsing before contraction of the ventricle, combined with a knowledge of the distance between the points of stimulation, permitted Erlanger to calculate the conduction velocity in the false tendon and to conclude that it was not less than 0 75 m/sec and was probably higher This conclusion showed that the atrioventricular delay could not be attributed wholly to slow conduction in the Purkinje system In the same paper Erlanger examined the possibility that the delay resulted from "something of the nature of a latent period" at one of three locations (1) the union of the atrial fibers with the fibers of the atrioventricular node, (2) the union of the fibers of the atrioventricular node with the fibers of the bundle of His, (3) the union of the Purkinje fibers with the ventricular fibers He pointed out that the transitions from node to His bundle and from Purkinje fiber to ventricle occur gradually and without apparent microscopic discontinuity He had moreover examined the third possibility directly and rejected it on the grounds that ' the reaction times to stimulation of a false tendon and of the heart wall directly have practically the same durations " He felt that Hering had insufficient evidence for assigning the delay to the fibers of the atrioventricular node and pointed out the great probability that the delay occurred as the result of a latency at the transition between atrial fibers and fibers of the atrioventricular node The work of the last two years has confirmed both the results and speculations of this remarkable paper in almost every detail

STUDIES OF SINGLE ATRIOVENTRICULAR NODAL FIBERS OF THE RABBIT HEART

Only recently has it been possible to record transmembrane potentials from single fibers of the atrioventricular node and to register simultaneously the action potentials of single fibers in adjacent atrium and His bundle (Hoffman Paes de Carvalho, and de Mello 1958, Hoffman Paes de Carvalho and Cranefield, 1958, Cranefield, Paes de Carvalho, and Hoffman, 1958 Hoffman and Cranefield, 1958*a*, Hoffman and Cranefield, 1958*b*, Cranefield and Hoffman, 1958*c*, Matsuda et al , 1958*a* *b*, Sano et al 1958, Cranefield et al., 1959, Hoffman Paes de Carvalho de Mello, and Cranefield 1959) These experiments have supplied some evidence on the exact site of

the normal atrioventricular delay, on the mechanisms of partial and complete heart block on the location of pacemaker sites and on the duration of the refractory period of nodal fibers In addition, records of the transmembrane action potentials obtained from single fibers at different locations in the node provide suggestive evidence for the mechanism of normal atrioventricular delay

Methods

The results described in this section were obtained in our laboratory, and many of them are previously unpublished A detailed description of methods therefore seems indicated Excised rabbit hearts were perfused with Tyrode solution through an aortic can-

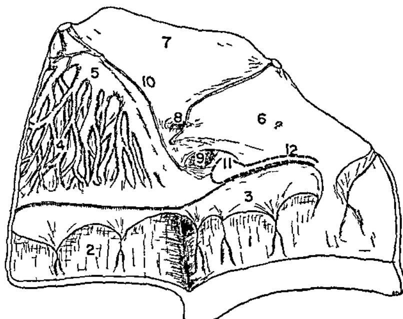


FIG 6-1 Schematic representation of the endocardial surface of the preparation of rabbit heart used for studies of the atrioventricular node The heavy dashes outline the approximate extent of the atrioventricular node (11) and bundle of His (12) The other areas designated by numbers are (1) interventricular septum (2) right ventricular wall (3) tricuspid valve (4) atrial appendage (5) crista terminalis (6) interatrial septum (7) superior vena cava (8) ostium of inferior vena cava (9) ostium of coronary sinus (10) sinoatrial node (*Hoffman Paes de Carvalho et al* 1959)

The Shape of the Action Potential

Transmembrane action potentials recorded from single fibers of atrium, atrioventricular node, and upper His bundle are shown in Fig 6-3 The atrial action potential (see Chap 3) is shown for comparison and the rapid upstroke marked reversal, high resting potential, and absence of depolarization during phase 4 should be noted

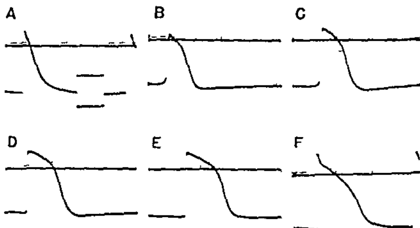


FIG 6-3 Transmembrane action potentials from single fibers of (*A*) atrium (*B*) upper node (*C*) (*D*) and (*E*) mid node and lower node (*F*) upper His bundle Upper trace represents line of zero potential and shows time calibration in intervals of 10 and 50 msec Voltage calibration in *A* of −50 and −100 mv (*Hoffman Paes de Carvalho et al* 1959)

These characteristics all stand in sharp contrast to the action potential recorded from a single fiber at the upper (atrial) end of the atrioventricular node (Fig 6 3*B*) At this location the resting transmembrane potential is low, and a variable amount of slow diastolic depolarization is present (see also Fig 6-4) The upstroke of the action potential shows either a clear prepotential (Fig 6-3*B*) or one or more notches (Fig 6-10*A*) and the rise time is greatly prolonged The overshoot, or reversal is either reduced or absent, and the peak of the action potential is rounded with no clearly marked phase 1 Finally, the time course of the voltage change during repolarization is markedly different from that of atrial muscle

Records obtained from single fibers 1 to 2 mm nearer the bundle of His (Fig 6-3*C*) reveal a gradual modification of the transmem

tion potentials The resting potential is greater, . . . upstroke becomes smoother and more rapid, and the reversal grows in amplitude Also, slow depolarization during phase 4 is more prominent still closer to the bundle of His records of the transmembrane potential show a partial transition to an action potential shape like that of peripheral Purkinje fibers with an increased velocity of depolarization during phase 0, prominent reversal and plateau and increased duration (Fig 6 3F) In the upper end of the bundle of His the resting potential is 90 mv or greater, the rapid upstroke terminates in a spiked reversal and phases 1, 2, and 3 are all clearly demarcated (Fig 6 2F, Fig 6 3F) The action potential duration is longer than at all sites nearer the atrium, and, during activity of an atrial pacemaker the level of membrane potential during phase 4 is constant

One additional fiber group must be described Between typical atrial muscle (Fig 6-4B) and the fibers thought to constitute the atrial margin of the atrioventricular node (Fig 6-4D) there are fibers which apparently show a stage of transition from one to the other Transmembrane potentials recorded from such fibers (Fig 6-4C) typically reveal a slight drop in the magnitude of the action potential and resting potential as well as some slowing of the rising phase and a change in the course of repolarization The duration of these action potentials is usually less than that of either atrial muscle or nodal tissue

Conduction Time through the Node

The spread of activity from the sinoatrial node to the bundle of His is shown in Fig 6-4 In this experiment the reference electrode was kept in a single fiber of the bundle of His, and the exploring electrode moved from the sinoatrial node through the atrium and atrioventricular node to another fiber in the His bundle The action potential shape at each location is similar to that described above In addition it can be seen even at the slow sweep speed employed that slow conduction is apparent only at the margin of the sinoatrial node and at the margin of the atrioventricular node An appreciable time is required for activity to spread from the sinoatrial pacemaker fiber to atrial muscle in the crista terminalis (Fig 6-4A and B) (Paes de Carvalho et al 1959) From this point to the atrial margin of the node spread is quite rapid (Fig 6-4B C, and D) Passage of the impulse through the atrial end of the atrioventricular

node is quite slow (Fig. 6-4D, E, and F), and by the time activity appears in the distal node (Fig. 6-4F and G) a major part of the atrioventricular delay has elapsed.

Records of this sort were also obtained, after removal of the normal sinoatrial pacemaker, from preparations driven with stimuli applied through a pair of electrodes located on the crista terminalis.

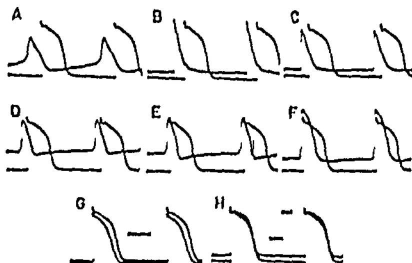


Fig. 6-4. Transmembrane action potentials recorded simultaneously from two locations in a preparation of rabbit heart. Lower trace in all records is from the same single fiber in the upper His bundle and serves as a time reference. Upper trace shows action potentials recorded from (A) sinoatrial node, (B) lower part of crista terminalis, (C) atrial margin of the atrioventricular node, (D) upper node, (E) mid node, (F) lower node, (G) transitional fiber, (H) His bundle. Time and voltage calibrations in G and H represent 200 msec and 100 mv. Note change in latency between nodal and His bundle activity from C to F. See text for discussion. (Hoffman, Paes de Carvalho, et al. 1959.)

In these experiments a drawing of the preparation was made from the vernier calibration of the horizontal movement of the micromanipulator carrying the exploring electrode. The conduction time to each recording site and the action potential shape at that point were plotted on the enlarged two dimensional scale drawing. From this record a graph of conduction time from atrium to His bundle could be constructed (Fig. 6-5).

Activity spreads through atrial muscle along the crista terminalis at a fairly rapid rate, and at an apparent conduction velocity of

0 8 to 1 0 m/sec In the His bundle where the action potential shape was similar to that of the peripheral Purkinje tissue, a similar value for conduction velocity was obtained Between these two regions

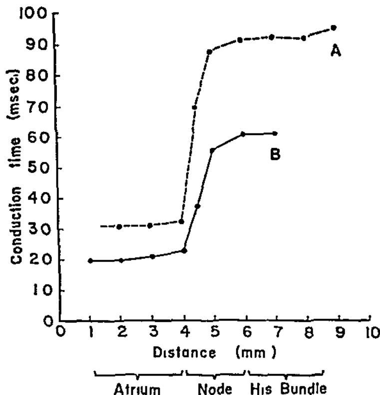


FIG 6-5 Graphs of conduction time across atrioventricular node from two experiments of the type shown in Fig 5-4 Zero is the time of application of a driving stimulus to the atrium See text for discussion (*Hoffman Paes de Carvalho et al* 1959)

conduction velocity was slower and as the graphs in Fig 6-5 indicate the greatest increase in conduction time appeared across a narrow zone at the atrial margin of the atrioventricular node In this region the action potentials show slow notched upstrokes or clearly marked prepotentials Attempts to calculate a conduction velocity in this zone from measurements made at points separated by 0 25 mm give a value of 0 05 m/sec or less Records obtained from

less widely separated points gave some indication of asynchronous activation of different fiber paths This observation suggests that the electrode may have been moved in a direction oblique to the actual spread of activity If this were true, the apparent linear

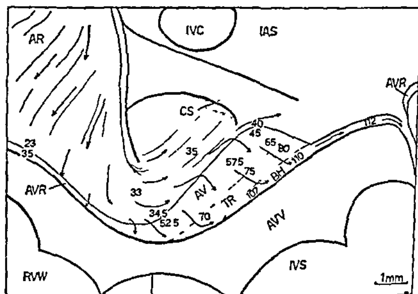


Fig 6-6 Diagrammatic representation of the spread of activity through the atrioventricular node of the rabbit heart Arrows indicate the direction of the spread of activity from stimulating electrodes located near the sinoatrial nod and numbers show conduction time in milliseconds to each location Other designations are as follows IVC inferior vena cava IAS interatrial septum AR atrial roof CS ostium of coronary sinus AV atrioventricular node TR transitional area (lower node) BH bundle of His AVR atrioventricular ring RVW right ventricular wall AVV atrioventricular valve IVS inter ventricular septum See text for discussion (*Unpublished data of A Paes de Carvalho*)

extent of the region of very slow conduction would be increased and the true conduction velocities would be even lower than calculated

Figure 6-6 is a diagram of the apparent lateral extent of the atrioventricular node and His bundle based on records of the transmembrane potentials of single fibers Also shown is the direction of the spread of activity in the node itself (Paes de Carvalho, personal communication) The representation is based on maps of the spread of excitation in rabbit hearts obtained in the manner just described

The noteworthy features are that activity appears to enter the node along a fairly broad front and proceeds from above downward toward the valve ring and that the fibers of the bundle of His collect in a funnel shaped exit from the lower margin of the node and then turn toward the interatrial septum Delay of a similar magnitude is seen at any point along the atrial margin of the node, activity appears more or less simultaneously in all fibers of the His bundle distal to the turn toward the septum

The Effects of Acetylcholine

The effects of acetylcholine on electrical activity of single fibers of the atrium atrioventricular node and His bundle are described at this point because they support the criteria employed in the preceding sections to identify various fiber types (Cranefield, Hoffman and Paes de Carvalho 1959) The possible mechanisms by which acetylcholine increases nodal delay or causes partial and complete block are discussed below in greater detail

If acetylcholine is added to a preparation of rabbit heart in a concentration sufficient to cause partial or complete atrioventricular dissociation, the changes in single-fiber activity differ markedly depending on the fiber type under the study It would be preferable, therefore to record simultaneously from multiple sites in atrium, upper and lower atrioventricular node and His bundle This has not yet been feasible instead records from one fiber type or the other have been paired with a common reference site and in this manner a comparison of the changes induced in different fibers by acetylcholine has been attempted Records of this sort showing the effects of acetylcholine at these sites are shown in Figs 6 7 to 6 9

Atrial Muscle In Fig 6 7 transmembrane action potentials of a single atrial fiber are shown on the upper trace and similar records from a fiber of the His bundle are shown on the lower trace Acetylcholine was added to the perfusion fluid after the control records in Fig 6 7*A* and caused partial and then complete block of transmission through the node The important aspects of this record are the marked shortening of the atrial action potential (Fig 6-7*B* through *F*) the appearance of complete block (Fig 6-7*E*), and the onset of pacemaker activity in the His bundle with failure of retrograde transmission (Fig 6 7*F*) It should also be noted that there are no changes in the action potential recorded from the bundle of His

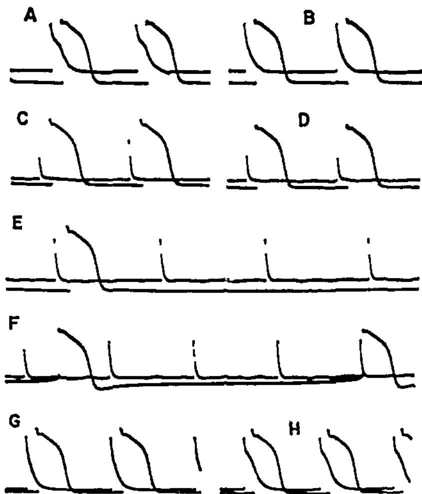


Fig 6-7 Transmembrane action potentials recorded from single fibers of rabbit heart showing the effect of acetylcholine on atrium (upper trace) and His bundle (lower trace) (*A*) Control (*B*) through (*F*) acetylcholine effect (*G*) and (*H*) recovery Note decrease in duration of atrial action potential (*B* *C*) complete atrioventricular dissociation (*E*) the onset of pacemaker activity in the His bundle without retrograde transmission to atrium (*F*) and the absence of changes in the His-bundle action potential other than those associated with pacemaker activity

other than those associated with the appearance of pacemaker activity (see Chap 7)

Lower Node Figure 6-8 shows a similar set of records from the same preparation In this case the reference electrode (lower trace) is in the same fiber of the His bundle and the exploring electrode is

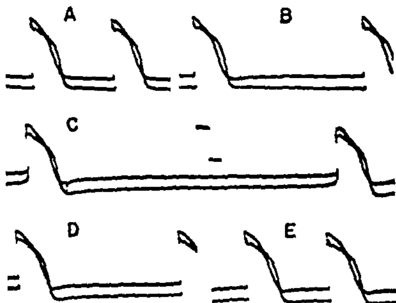


FIG 6-8 Transmembrane action potentials recorded from the same preparation as shown in Fig 6-7 Upper trace single fiber in the lower part of the atrioventricular node lower trace single fiber in the bundle of His (*A*) Control (*B*) through (*D*) acetylcholine effect (*E*) recovery Throughout this experiment the change in atrial rate was comparable to that shown in Fig 6-7 Note that except for the onset of pacemaker activity in the bundle of His there are no apparent changes in the transmembrane potentials recorded from either site Also during His-bundle escape there is no block of transmission from His bundle to the lower part of the atrioventricular node

in a single fiber of the lower part of the atrioventricular node After registration of the control records shown in Fig 6-8*A*, acetylcholine was again added in the same concentration as that employed for Fig 6-7 Again complete block of atrioventricular transmission was produced and as the record clearly demonstrates the site of failure of transmission is above the recording electrode in the lower node The nodal fiber under study shows some slowing (Fig 6-8*B*) the

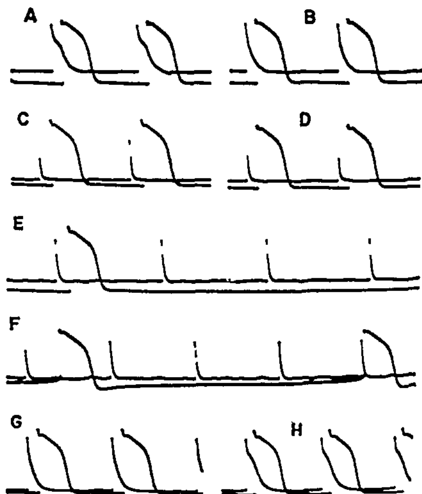


Fig 6-7 Transmembrane action potentials recorded from single fibers of rabbit heart showing the effect of acetylcholine on atrium (upper trace) and His bundle (lower trace) (*A*) Control (*B*) through (*F*) acetylcholine effect (*G*) and (*H*) recovery Note decrease in duration of atrial action potential (*B C*) complete atrioventricular dissociation (*E*) the onset of pacemaker activity in the His bundle without retrograde transmission to atrium (*F*) and the absence of changes in the His-bundle action potential other than those associated with pacemaker activity

change in the level of resting potential of the nodal fibers At other sites lower in the node or in the bundle of His the only changes in the transmembrane potentials during block due to acetylcholine are those associated with changes in rhythm and the development of

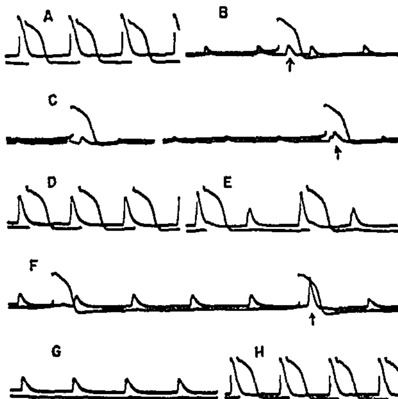


FIG 6-9 Upper trace single fiber of the atrioventricular node lower trace single fiber of the bundle of His (*A*) Control (*B*) and (*C*) total atrioventricular block after the introduction of acetylcholine with pacemaker activity in the bundle of His (*E*) Further effect of acetylcholine 2 1 block The nodal action potential which appears during the His-bundle action potential in *F* is discussed in the text *G* shows total block and *H* shows recovery (*In part from Cranefield Hoffman and Paes de Carvalho* 1959)

slow depolarization during phase 4 Finally the pacemaker site in each experiment is in a fiber of the bundle of His rather than in the lower part of the atrioventricular node

The records shown in Fig 6-10 supply additional pertinent infor-

mation In this preparation one electrode is again located in a fiber of the bundle of His (lower trace) and the other in a fiber near the atrial margin of the atrioventricular node (upper trace) The action potential of this particular nodal fiber shows several notches on the *upstroke as well as a suggestion of hyperpolarization between phases*

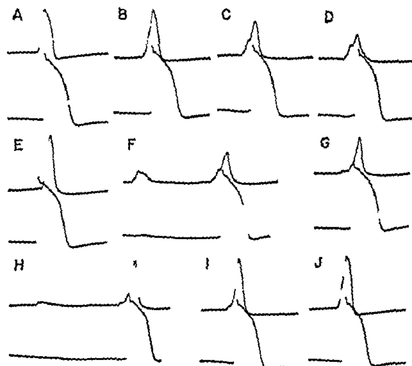


Fig 6-10 Upper trace single fiber of the atrioventricular node lower trace single fiber of the bundle of His (*B*) through (*I*) show the effect of acetylcholine Note the extreme degree of separation of the upstroke into three components in *D* (*Cranefield Hoffman and Paes de Carvalho* 1959) See text for discussion

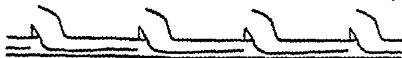
3 and 4 After the addition of a lower concentration of acetylcholine than in the previous experiment the nodal action potential again *decreases in amplitude and 2 1 block appears (Fig 6-10F)* Of particular interest is the increased prominence of the notches on the rising phase (Fig 6-10*B C D*) and their separation Thus, only two components are seen in the first nodal response in Fig 6-10*F*, and in Fig 6-10*H*, only one Several other aspects of these records should

be emphasized First, the altered time relationship between depolarization of the nodal fiber and the action potential of the His bundle suggests that activity of the latter results from excitation traveling over different paths Also, the His bundle action potential in Fig 6-10E results from activity of an infranodal pacemaker and propagating in a retrograde direction, elicits a full sized action potential from the nodal fiber More important, the rising phase of that particular nodal response is free of notches and the action potential is of somewhat shorter duration than the control The records in Fig 6-10I and J show a return to normal activity which is associated with the reappearance of notches on the rising phase of the nodal action potential

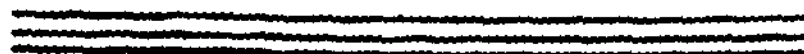
The records obtained from this preparation again demonstrate that acetylcholine induced failure of nodal transmission occurs in fibers located at the atrial margin of the node Moreover although the response of the nodal fiber to atrial activity consists only of small depolarizations the same fiber is excitable and capable of generating a normal action potential in response to retrograde transmission from the bundle of His The disappearance of notches on the rising phase of the nodal action potential under this condition will be discussed subsequently However the fragmentation of the nodal response (Fig 6-10B through H) and the notched upstroke of action potentials recorded from fibers of this type strongly suggest that normal excitation results from the confluence of several excitatory events

Atrionodal Junction One additional fiber group must be discussed in relation to the effects of acetylcholine on nodal transmission These fibers which are thought to represent atrial muscle just at the atrionodal junction (see Fig 6-4C) are particularly difficult to locate and also are quite difficult to record from When it has been possible to record continuously from a single fiber of this type before during and after atrioventricular block caused by addition of acetylcholine to the perfusate the records of the transmembrane potential show several characteristic changes (Fig 6-11) In the presence of acetylcholine fibers of this type show an acceleration of the initial phases of repolarization a marked decrease in amplitude, and some prolongation of the latter part of phase 3 It should be noted that with the same or higher concentrations of acetylcholine other types of atrial fibers in the same preparation show a decrease in action poten-

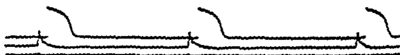
tial amplitude of at most a few millivolts Another effect sometimes noted in these particular fibers is a partial separation of the action potential into two components, one synchronous with activity of adjacent atrial muscle and the other related in time to activity of



A



B



C

FIG 6-11 Transmembrane potentials recorded from the bundle of His (upper trace) and atrial fiber at the atrionodal junction (middle trace) and a bipolar electrogram recorded from atrial muscle (lower trace) Segments of a continuous record showing (*A*) control (*B*) acetylcholine induced arrest (*C*) partial recovery from acetylcholine effect See text for discussion

the node and His bundle (see Fig 6-16) These particular fibers are discussed more fully in a subsequent section However, it is apparent from simultaneous records of activity in atrium in this fiber group and in the His bundle that there is no large delay between atrium and these fibers either during normal or retrograde conduction Also, the changes described for these fibers are not invariably associated with a decrease in the level of resting potential

The Effects of Rate and Rhythm

Changes in rate are known to influence the magnitude of atrioventricular delay and to cause partial or complete failure of atrioven

tricular transmission These effects have been studied by recording from single fibers at various locations in atrium, atrioventricular node and His bundle In some experiments the preparation has been constantly exposed to a low concentration of acetylcholine, and the effects of rate changes have been superimposed thereon Rate-induced changes in the transmembrane of potentials are described in this section the mechanism of partial and complete block is discussed below

Sudden Acceleration Figure 6-12 shows records from a single fiber in the atrial part of the atrioventricular node (upper trace) and upper His bundle (lower trace) After registration of two action

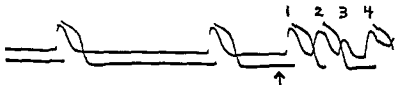


FIG 6 12 Transmembrane potentials recorded from single fibers of rabbit heart Upper trace atrioventricular node lower trace bundle of His Rate increased by driving stimuli at arrow Note changes in nodal action potentials 2 3 and 4 delay in activation of His bundle by 2 and failure of excitation by 3 Microelectrode in nodal fiber dislodged after response 4 See text for discussion

potentials at the control rate the frequency was suddenly increased to a new steady value (arrow) and as can be seen only three of the succeeding four atrial responses propagate to the bundle of His The cause of the partial failure of transmission can be appreciated after inspection of the record of nodal activity The first nodal response after a somewhat abbreviated diastole is normal in amplitude and duration The next however starts just before the completion of the preceding phase 3 and shows not only a decrease in rising velocity and amplitude but also an increase in duration The corresponding excitation of the His bundle is considerably delayed The third nodal response beginning considerably before completion of repolarization shows a marked diminution both in amplitude and duration and fails to propagate to the bundle of His Presumably because of the short duration of this response the next atrial action potential gives rise to a nodal response of normal amplitude which excites the bundle of His after a normal delay This sequence with failure of nodal transmission of every third atrial response was maintained for the duration of the tachycardia

Two aspects of the records shown in Fig 6-12 deserve special emphasis First, failure of the normal mechanisms of excitation appears in fibers located in the atrial part of the node Second, the response of the nodal fiber appears to depend more on the duration of the preceding phase 4 than on the level of membrane potential at the time of excitation (see nodal responses numbered 2, 3, and 4)

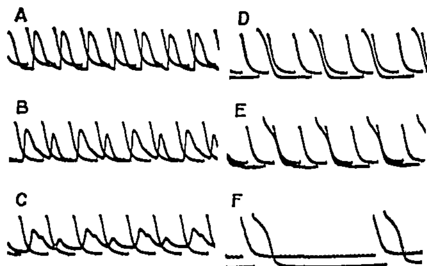


FIG 6-13 Transmembrane potentials recorded from single fibers of atrium and from fibers of atrioventricular node and bundle of His of rabbit heart showing the changes in transmission of activity during sustained tachycardia (*A*) Atrial margin of node (*B*) and (*C*) nodal fibers distal to those in *A* (*D*) lower node (*E*) bundle of His The same atrial fiber is employed for records *A* through *E* The records in *F* are included to show the magnitude of delay between atrium and bundle of His at a slower rate See text for discussion

Maintained Tachycardia The records in Fig 6-13 show the response of fibers located in atrium upper and lower atrioventricular node, and bundle of His during a sustained tachycardia The first five records show a progressive failure of transmission of alternate impulses and complete 2 1 block in the lower node and His bundle The alternate failure of transmission appears to result from action potentials which arise from nodal fibers prior to the end of repolarization and which in turn fail to excite more distal fibers The increased delay in transmission is associated with a decrease in the rise velocity and amplitude of the nodal action potentials The notch

on the descending limb of the nodal action potentials (Fig 6-13C) is synchronous with activity in the lower node and His bundle

Acceleration in the Presence of Acetylcholine In a number of experiments the effects of tachycardia were superimposed on those of acetylcholine, or acetylcholine was added to preparations which were already being driven at a high rate In general the combined effect of these two influences was to increase nodal delay and to enhance failure of nodal transmission by acting on the atrial fibers at the atrionodal junction and on the nodal fibers at the atrial margin of this structure The primary changes at each site were similar to those caused by acetylcholine or rate alone In no instance was there any change in the transmembrane action potentials recorded from fibers in the lower node or bundle of His other than those previously described

Identification of Fiber Groups

Some justification of the terms used to identify the various fiber types is required The first criterion employed was the anatomical relationships of each fiber Identification of the bundle of His presents no particular problem because of the compact nature of this structure and the large fiber size The shape of the action potential, which greatly resembles that of peripheral Purkinje fibers, the high conduction velocity, the presence of typical pacemaker activity, and the insensitivity to acetylcholine all support the contention that these fibers constitute the His bundle

The fibers identified as belonging to the lower part of the atrioventricular node have been identified on the following bases They are in direct continuity with His bundle below and node above, they have an action potential which is intermediate in shape between that of node and His bundle and they are the only group of fibers through which activity normally spreads to the bundle of His It might be preferable to classify this fiber group as a type intermediate, or transitional between atrioventricular node and His bundle and to do so would be of no importance to the results or interpretations and might agree somewhat better with microscopic anatomy The decision to identify them as lower nodal fibers is based largely on use of the terms *upper* (or atrial) and *lower* (or ventricular) node by earlier workers (Monckeberg 1921)

The fibers identified as belonging to the upper or atrial part of

the atrioventricular node have been identified in the first place on the basis of gross anatomical location In addition, it is only through these fibers that a continuous sequence of excitation from atrium to ventricle can be traced Furthermore it is during transmission through these fibers that a major part of the usual atrioventricular delay elapses Partial or complete atrioventricular block occurs in this group of fibers whether it is caused by acetylcholine high rate, anoxia (Hoffman, Cranefield and Paes de Carvalho, 1959), or a combination of these factors Also, the transmembrane action potentials recorded from these fibers are distinctive They differ greatly from records of atrial fibers, and they differ also from lower nodal fibers in the notched, slow upstroke of the action potential, the amplitude of the resting and action potential, and the changes induced by acetylcholine or high rate

A final group of fibers has been described These are the atrial fibers just proximal to the atrionodal junction They have been classified with atrium rather than with node for the following reasons The transmembrane action potential is similar to that of atrial fibers and shows a decrease in duration in the presence of acetylcholine During normal transmission these fibers are excited before the atrioventricular delay and during retrograde transmission after a major portion of the ventriculo atrial delay As with the other fiber groups although histological identification has not been made, this is of little importance in the absence of any exact correlation between previous descriptions of microscopic anatomy of this region and the physiological identification of the atrioventricular node These fibers are included with atrial muscle because this terminology simplifies description of delay and block in the subsequent sections and suggests a possible correlation between structure and function

The Mechanism of Atrioventricular Delay

The results obtained from studies of single fibers in atrium atrioventricular node and His bundle of the rabbit heart do not permit a conclusive statement concerning the mechanism of delay However the experiments described above strongly suggest certain interpretations The transmembrane potentials recorded from single fibers in a narrow zone at the atrial margin of the node have a low resting potential and a slowly rising action potential of low amplitude All these factors might be expected to result in a diminished

conduction velocity Actual measurements of conduction time from atrium to His bundle show that slowing is localized to a narrow zone and that the action potentials recorded from this zone are of the type described Under control conditions no marked discontinuities of transmission through this area have been detected It seems reasonable to conclude therefore, that the normal atrioventricular delay is due to slow conduction over a short distance in these particular fibers A calculated conduction velocity from data similar to that of Fig 6-5 gives a value of 0 05 to 0 02 m/sec over a distance of 1 mm This is not incongruous with the calculations Lewis (1925) made for dog heart in which he assumed a velocity of 0 2 m/sec over a distance of 10 mm, and is in good agreement with calculations made by Scher et al (1959) on the basis of unipolar electrograms recorded from perfused dog heart

It seems therefore that the normal atrioventricular delay results from very slow spread of excitation over a short distance The cause of this slow spread remains to be determined However, the likelihood of conduction at normal velocity over long tortuous paths can be disregarded as can other hypotheses summarized in the beginning of the chapter Results of our studies on rabbit and dog heart (see below) have suggested that the normal delay is due to slow conduction which may be decremental Matsuda (personal communication) has obtained somewhat different records from studies of dog heart and has concluded that the delay is the result of both the slow conduction and delay in depolarization (see below)

Evidence for Decremental Conduction By decremental conduction we may understand a type of conduction in which the properties of the fiber change along its length in such a manner that the action potential becomes progressively less effective as a stimulus to the unexcited portion of the fiber ahead of it The change may progress to the point where conduction fails completely or the properties may again become more favorable to propagation Since the efficiency of the action potential as a stimulus depends upon its amplitude upon its rate of depolarization upon the extent to which the depolarization caused by it reaches ahead and upon the threshold of the fiber a progressive change in any of these factors might cause decremental conduction The propagation of an impulse decrementally differs from the electrotonic spread of an impulse into an inexcitable fiber in that in decremental conduction at the point at which

block occurs the fiber is excitable but the action potential is unable to excite it

In general a change in fiber properties in the direction of causing decremental conduction (e g, progressively increasing threshold decreasing space constant, decreasing action potential amplitude decreasing rate of depolarization) is also a change which will cause progressively slower conduction The observation that action potentials in the atrioventricular node show a low rate of depolarization a low amplitude, and a low propagation velocity naturally suggests the possibility that conduction in nodal fibers is decremental This possibility is strengthened by the observation that during increased atrioventricular nodal delay caused by acetylcholine or high rate the action potentials become still lower in amplitude and rate of depolarization and in fact do fail to propagate through the node

There is no demonstrative evidence to support the interpretation that conduction in the atrial portion of the atrioventricular node is in fact decremental However the hypothesis fits all the observations noted below and is contradicted by none of them A key observation is that nodal fibers are excitable even during total block

Characteristics of Transmembrane Potentials in the Atrial Part of the Atrioventricular Node

1 Low resting potential
2 Low amplitude and overshoot
3 Very low rate of depolarization
4 Very low conduction velocity
5 Diminished amplitude with acetylcholine or high rate
6 Diminished rate of rise with acetylcholine or high rate
7 Failure of conduction with acetylcholine or high rate
8 Failure of conduction without inexcitability (see Fig 6-10*L*)
9 Slurred upstroke with steps or notches
10 Increased notching of upstroke with acetylcholine
11 Alternation in amplitude with acetylcholine or high rate
12 Disappearance of notches during retrograde conduction
13 Temporal dispersal of steps on the upstroke is associated with diminished amplitude
14 Notches on upstroke may separate into several *discrete* depolarizations
15 Temporal summation of depolarizations is apparent and may result in excitation

The first eight of these properties are compatible with the concept that the fibers at the atrial margin of the atrioventricular node conduct decrementally The remaining observations noted above are concerned with fragmentation of the response of these fibers into more than one event and bear on another aspect of nodal activity They do not negate the concept that conduction is decremental but do require a further independent interpretation

Anatomical Considerations The observation that action potentials recorded from the atrial margin of the atrioventricular node show steps or notches on the upstroke and that these notches are either accentuated by the action of acetylcholine or separated into discrete depolarizations by acetylcholine or high rate suggests that the excitatory process consists of more than one event The general idea is that there are two or more excitatory events in a given nodal fiber and that these events are somewhat unequally affected by acetylcholine and in particular are separated temporally This idea permits an explanation of observations 8 through 15 and must be valid in a general way no matter what the anatomical or physiological explanation of the separateness of the events may be Since however it is extremely difficult to describe a theoretical explanation of these steps without an anatomical picture in mind separate explanations will be offered for each of the two most probable anatomical mechanisms These mechanisms are first that separate excitatory impulses arrive at a common point along separate converging pathways and second that a single fiber presents obstacles to the smooth progress of the impulse

The first mechanism might result anatomically either from the coalescence of several small fibers into one large one or the impingement of several small fibers upon another fiber with which they are not in cytoplasmic continuity The diagram in Fig 6 14 represents the first of these possibilities and presents several advantages Descriptions of the microscopic anatomy of the node (Tawara 1906 Monckeberg, 1921) all agree on several points first that there is a marked decrease in diameter of atrial fibers just above the node second that fibers in the atrial part of the node are small in diameter show profuse interconnections and are closely intermingled with fine atrial fibers and finally, that there is an increase in fiber diameter and decreased branching as one progresses through the lower node to the bundle of His Figure 6 14 shows the action poten

tials which presumably are recorded at each site during normal transmission and also shows the recorded changes at each site under the influence of acetylcholine or high rate It is apparent that most

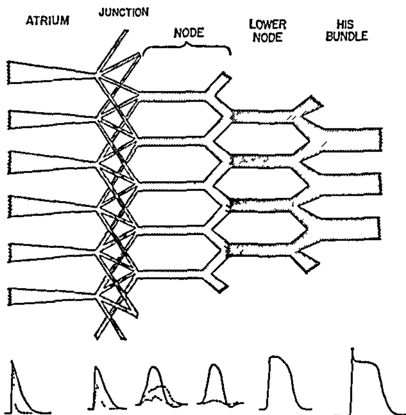


FIG 6-14 Schematic representation of possible anatomical relationships between fibers of atrium atrioventricular node and bundle of His and action potentials recorded from each location under normal conditions (solid lines) and under the influence of acetylcholine (dotted lines) See text for discussion

of, if not all, the observations made during studies of the rabbit heart are compatible with this diagram

There is at present no direct evidence either from physiological or electron microscopic observations to support the possibility of an interruption of cytoplasmic continuity at any point between atrium and ventricle Neither do embryologic studies (Patten 19‿6) give

evidence of this sort Long delays may occur at the junction of Purkinje fibers with papillary muscle during retrograde transmission (see Chap 7) and there has been a clear demonstration of cytoplasmic continuity at that junction (Kugler and Parkin, 1956) Thus there seems to be little need to assume interruption of cytoplasmic continuity as an explanation of atrioventricular nodal activity The other major possibility that a single fiber might present obstacles to the smooth progress of conduction cannot be adequately evaluated at this time It is possible that the presence of intercalated disks might have this effect However, studies of the longitudinal resistance of other types of cardiac fibers have failed to reveal any discontinuous contribution which might be due to the presence of such disks (Weidmann 1956*a*) A clear statement of the mechanism of nodal delay must await studies of the electron microscopic histology and passive electrical properties of nodal fibers

The Effect of the Low Resting Potential of Nodal Fibers Records of the transmembrane potentials of single fibers at the atrial margin of the atrioventricular node regularly show a low resting potential While it is possible that this low value results from injury to the fiber or from improper impalement the uniformity of this observation (see Hoffman Cranefield and Paes de Carvalho 1958 1959, Cranefield Hoffman and Paes de Carvalho 1959 Matsuda et al, 1958*a* *b*) in a variety of preparations support its reality Information is not yet available which would permit an evaluation of the contribution of this low resting potential to slow atrioventricular conduction Studies of other fibers and in particular of Purkinje fibers (Weidmann 1955*a*) indicate that a low resting potential may cause a slowly rising action potential of reduced amplitude which propagates at reduced velocity This certainly is the result when a fiber is stimulated before the end of phase 3 (see Chap 8) or after partial depolarization by KCl It has not yet been possible to increase the resting potential of nodal fibers by electrical polarization and thus study the effect of this procedure on the action potential and conduction velocity However a pertinent experiment suggested by early studies of decremental conduction in heart (Drury, 1925–1926 Schmitt and Erlanger, 1928–1929) has been performed on papillary muscle Figure 6-15 shows records obtained from single fibers of papillary muscle before and after exposure to excess KCl During recovery from K^+ induced de-

polarization it can be seen that the large depolarization recorded near the stimulating electrode appears at the distal recording site either, when the resting potential is low, as an electronically decremented potential or, at a somewhat higher resting potential, as an active response which shows one or more notches during phase 0

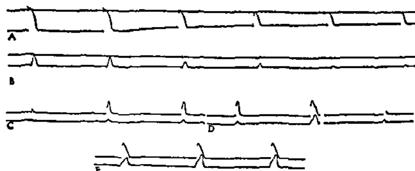


FIG 6-15 Transmembrane potentials recorded from isolated papillary muscle at some distance from the site of the driving electrodes showing (*A*) one control action potential and then rapid depolarization caused by excess extracellular KCl until (*B*) only small decremented potentials are apparent at the recording site In *C* *D* and *E* two microelectrodes have been used to record activity near (top trace) and farther from (bottom trace) the site of the drive electrodes In *C* and *D* the strength of the stimulus is varied to produce responses of varied amplitude at the electrode site note the configuration of the second response at the distal electrode in *D* In *E* at a slightly higher level of membrane potential responses at the distal recording site show a prolonged step terminating in the upstroke of the local action potential See text for discussion

This experiment demonstrates several points First decremental conduction can occur in cardiac fibers and second, a low resting potential and some inhomogeneity of excitability can cause slurred notched upstrokes and appreciable local delay Thus, while supporting the general concept for the mechanism of delay it rules out an absolute requirement for any special underlying anatomical arrangement for the occurrence of one or two notches on the upstroke Other results from studies of nodal fibers however appear to require some consideration of the special anatomy of this structure

Partial and Complete Atrioventricular Block

The activity of nodal fibers during partial and complete failure of nodal transmission has been shown in several of the preceding figures In each case the failure of the excitatory event appeared to

be localized to fibers at the atrial margin of the atrioventricular node At this time it is desirable to discuss several aspects of these records in greater detail

The Role of Atrial Fibers at the Atrionodal Junction In the presence of acetylcholine or when acetylcholine is combined with high rate it has been seen in some experiments that the action potential recorded from atrial fibers just above the atrioventricular node decreases not only in duration but also in amplitude It is assumed that this change in electrical activity is responsible for the asynchronous and therefore ineffective activation of the nodal fibers near the atrial margin Whether or not there is also a simultaneous change in threshold of the e nodal fibers has not been determined, however it can be inferred from records like those in Fig 6-9 and Fig 6 10 that the thre hold potential is low at this time Under these conditions therefore decrement is increased to the point of block at the upper nodal fibers at least in part because of the decrease in action potential amplitude of the atrial fibers located just proximal to the junction

The Refractory Period of Nodal Fibers Many early explanations of altered transmission through the atrioventricular node were based on a supposed long refractory period of nodal fibers Certain of the records obtained at high rates of stimulation are in agreement with this concept The failure of transmission in these cases is again localized to fibers in the atrial part of the node but appears to result from the maintained long duration of the action potential in spite of the increased rate Moreover in some instances it appears that the delayed transmission when effective in exciting lower nodal tissue may actually prolong the action potentials recorded from the atrial part of the node (see Figs 6-13 6-16) Finally records like those in Fig 6-17 suggest that in fibers in the atrial part of the atrioventricular node as in some fibers of the sinoatrial node (see Chap 5) recovery of excitability may lag behind repolarization to a greater extent than in other types of cardiac fibers (see Chap 8)

Retrograde Transmission and Ventriculo atrial Delay In most of the records from preparations of rabbit heart retrograde transmission was seen only in the presence of acetylcholine However, when normal transmission from atrium to node failed the point of block was in the atrial part of the node If at this time a pacemaker in the bundle of His began firing retrograde transmission was unimpaired

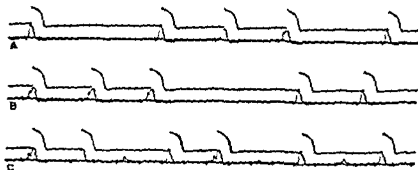


FIG 6-16 Tran_membrane potentials recorded from the bundle of His (upper trace) and upper atrioventricular node (middle trace) and bipolar electrograms from atrial mu_cle of rabbit heart showing *changes in the electrical activity of* nodal fibers induced by rate changes in the presence of low concentrations of acetylcholine (*A*) The effect of a bri f period of acceleration, (*B*) the record obtained during and after a 2 1 block (*C*) varying block a a higher atrial rate Note changes in the delay between activity in atrium and bundle of His as well *as the changes in the shape and ampl tude of the nodal action potentials* In all records the second component of the nodal response is noted only in synchrony with activity in the lower nod

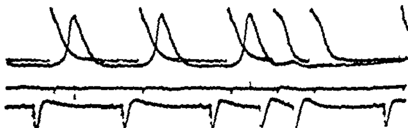


FIG 6-17 Records from rabbit heart showing tran_membrane potentials of single fibers of atrium (top trace) and atrioventricular node (econd trace) a bipolar electrogram from the bundle of His (third trace) and a unipolar electrogram from the sinoatrial node region (bottom trace) Note that the bipolar electrogram shows some low voltage polypha_ic activity synchronous with atrial activity and a sharp high voltage complex due to activity of the bundle of His After three normal driven beats a pair of extrasystoles excite atrial muscle but elicit only minimal depolarization of the fully repolarized nodal fiber and thus fail to excite the bundle of His

in the lower node If ventriculo-atrial block was pre ent failure of excitation again was localized to the atrionodal junction Under normal conditions the delay in transmi_sion from His bundle to atrium was similar in magnitude to that seen during normal conduction and was al_o localized to the atrionodal junction

STUDIES OF DOG HEART

The first published records of electrical activity of single fibers of atrioventricular nodal tissue in perfused puppy hearts are reproduced in Fig 6-18 (Matsuda et al 1958a b) During normal trans-

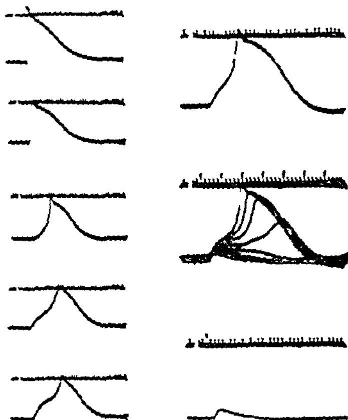


FIG 6-18 Transmembrane potentials recorded from single fibers of the dog heart. The records in the left column are obtained from different areas in the atrium (top record) and in the atrioventricular node The records in the right column show a control nodal action potential (top) several superimposed sweeps during the onset of acetylcholine effect (middle) and complete failure of transmission from atrium to node (bottom record) In all records the top trace is the line of zero potential and shows time marks at intervals of 10 and 50 msec The voltage calibration in all records represents 100 mv See text for discussion (*Matsuda et al* 1958a b)

mission the nodal action potential arises out of a long prepotential, the duration of this initial step is increased by acetylcholine and decreased by epinephrine These records have been interpreted as showing the normal mechanism of atrioventricular delay to depend

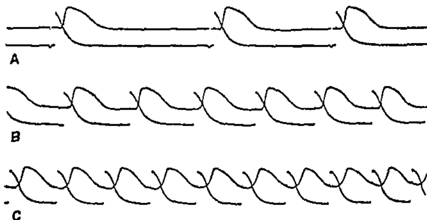


Fig 6-19 Transmembrane potentials recorded from atrioventricular node (upper trace) and atrium (lower trace) of puppy heart showing the decrease in amplitude and rising velocity of the nodal action potential observed at high rates of stimulation

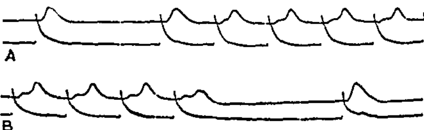


Fig 6-20 Transmembrane action potentials recorded from atrioventricular node (upper trace) and atrium (lower trace) of puppy heart in the presence of low concentrations of acetylcholine Note that during acceleration the nodal response is preceded by a single step of long duration similar to that shown in Fig 6-18 See text for discussion

in part on slow conduction and in part on the delay *resulting from* the single slow step Our own studies of dog, puppy, and rabbit hearts suggest a different interpretation As can be seen in Figs 6-19 and 6-20, records from isolated preparations of dog and puppy hearts show that action potentials of atrium and atrioventricular node are

similar to those shown for rabbit heart Under the influence of increased rate the nodal action potentials may show only a decreased rising velocity and amplitude (Fig 6-19) However, when the safety factor of conduction has been greatly decreased by addition of acetylcholine (Fig 6-20) with the onset of rapid rates the nodal potentials are seen to arise from a single long prepotential

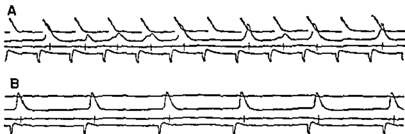


FIG 6-21 Records from rabbit heart showing changes in activity of nodal fibers induced by high atrial rates The top trace shows transmembrane potentials from a single atrial fiber close to the node and the second trace shows transmembrane potentials from a single fiber in the atrioventricular node The third trace is a bipolar electrogram recorded from the bundle of His and shows an initial low voltage complex representing activity in the atrium and a sharp high voltage complex due to activity of the bundle of His (retouched) The bottom trace is a unipolar electrogram recorded from the region of the sino-atrial node In *A* when the atrium is driven at a rapid rate the nodal response varies in amplitude and often arises from a single step of long duration In all cases the major deflection of the nodal response is almost simultaneous with propagated activity in the bundle of His In *B* the atrial rate is lower and the nodal action potentials have resumed a normal configuration In this record the atrial microelectrode has become dislodged from the atrial fiber under study

A similar type of record has also been obtained from rabbit hearts In one such experiment in addition to transmembrane records from atrium and upper node unipolar electrograms were recorded from atrium and His bundle (Fig 6 21) These records show that at times the large depolarization of the nodal fiber is either synchronous with or subsequent to firing of the His bundle the long slow step on the other hand is comparable in duration to the atrio-His bundle delay While delay in nodal transmission may be caused by a delay of this type under certain conditions it is likely that in many instances such records show failure of normal excitation of the fiber under study The large depolarization in such cases results from depolarization distal to the fiber from which the transmembrane potential

is recorded or, in some cases, from retrograde excitation which may take place It is felt that during normal atrioventricular transmission in dog as well as in rabbit there is no actual interruption in the spread of excitation Studies of another type (Scher et al , 1959) support this view

Another study of the electrical activity of single fibers of the atrioventricular node of the dog heart has been reported by Sano (Sano Tasaki et al , 1958) In this study the normal action potential of the atrioventricular node showed a clear step on the rising phase similar to that shown in our records Also regional differences in the shape of the action potential were observed although no attempt was made to relate these differences to the spread of activity through the node During atrioventricular block the changes in transmembrane potentials of nodal fibers were similar to those described for rabbit hearts The addition of quinidine procaine amide and G strophanthin in apparently high concentrations was shown greatly to increase the duration of the initial step of the action potential and produce a slowing of the rising phase and diminished reversal

OTHER SPECIAL PROPERTIES OF ATRIOVENTRICULAR CONDUCTION

Several peculiarities of atrioventricular transmission which have recently been studied by means of other techniques can be interpreted at least in part in terms of the results obtained from studies of the electrical activity of single nodal fibers Certain of these are discussed in this section

Evidence for a Dual Atrioventricular Transmission System

Moe (Mendez et al 1956 Moe et al 1956) and more recently Rosenblueth (1958b) have presented evidence to support the concept that there are two pathways connecting the atria and ventricles and that these two paths have different functional properties One which conducts at a higher velocity has a longer functional refractory period than the other more slowly conducting path The basic experimental demonstration of a dual atrioventricular conducting system is obtained by varying the interval between driving and testing stimuli applied to the atrium As the testing stimulus is applied earlier and earlier in the driven cycle, the interval between ventricular responses at first shortens in direct proportion to the

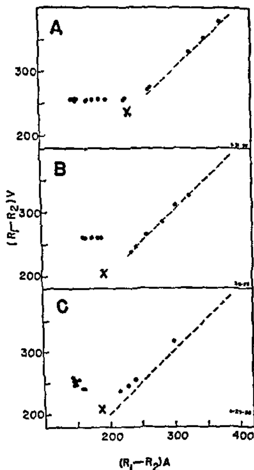


FIG 6-22 *Plot of the interval between regularly driven responses and premature responses in the atria* $(R_1 - R_2)_A$ in relation to the corresponding ventricular intervals $(R_1 - R_2)_V$ of dog heart Three examples from different experiments (*A*) Heart-lung preparation (*B*) dog with stellate ganglia excised (*C*) intact dog See text for discussion (*Moe et al* 1956)

drive-test interval However when the drive-test interval is less than a certain limiting value the interval between ventricular responses remains unchanged This limiting value is usually thought to depend upon the duration of the functional refractory period of the atrioventricular conducting system (Fig 6 22*A*) This is the

type of response which has been observed most frequently by a number of investigators In contrast, in a certain proportion of animals (dogs, goats, turtles) Moe observed a different series of events (Fig 6 22B, C) At drive test intervals shorter than the functional refractory period of the atrioventricular conducting system there appeared either an abrupt (Fig 6 22B) or progressive (Fig 6-22C) increase in the delay between ventricular responses to drive and test stimuli This change in atrioventricular conduction time has been attributed to transmission through a slow path with a short functional refractory period (Moe et al, 1956) A similar abrupt change in conduction time has been observed during transmission of premature beats from ventricle to atrium (Moe et al 1956, Rosenblueth 1958b) Also various experimental procedures have been shown to alter the response pattern from that seen in Fig 6 22A to that of either Fig 6 22B or C This phenomenon has been observed in heart lung preparations (Moe et al 1956) and in in situ hearts following vagotomy excision of the upper thoracic sympathetic chains and adrenal ligation (Rosenblueth 1958b)

A considerable amount of indirect evidence has been presented in support of the existence of a dual conduction system Moe has shown that activity which presumably reaches the ventricle over the rapid system causes a different sequence of activation than that traversing the slower path Also atrial and ventricular echoes have been explained on the basis of intercommunications between the two pathways (Moe et al 1956 Rosenblueth 1958c) A detailed analysis of all available evidence supporting this hypothesis can be found in the original communications Anatomical identification of two separate pathways has not been provided It is uncertain whether or not the postulated dual conduction system is localized to the atrioventricular node or extends to the periphery of the ventricular Purkinje system (Moe et al, 1956) Moreover it should be emphasized that several aspects of the change in atrioventricular transmission time might be explained without assuming the participation of a dual system The usual observation (Fig 6 22A) is encountered even at the simple junction between ventricular muscle and Purkinje fibers during retrograde transmission of premature beats (see Fig 8-25) An abrupt change in latency of response in the Purkinje system with increasing prematurity of ventricular action potentials may also be observed (Fig 8-24) A change in atrioventricular transmis-

sion time similar to that shown in Fig 6-23C can result from changes in electrical activity of the fibers at the atrial margin of the atrioven tricular node The increasingly long latency of ventricular response in this case may result from progressively slower propagation of the premature responses not only in the node but also in the bundle of His The major difference between the two response patterns (Fig 6-23A and C) thus depends primarily on the duration of the "func tional refractory period at the atrial margin of the node If this is longer than any other between atrium and ventricle the response pattern will be similar to that in Fig 6-22A If there is a gradient of increasing duration of the functional refractory periods through the node and bundle of His however the response pattern will be that shown in Fig 6-22C The ease with which the pattern changes from one type to another can be understood if one recalls the marked change in electrical activity of nodal fibers and particularly those at the junction of atrium and node which are brought about by a variety of factors such as acetylcholine epinephrine and hypoxia

The explanation just given for changes in atrioventricular con duction time of premature beats does not explain an abrupt increase in latency such as that shown in Fig 6 22B To date we have not obtained any direct experimental demonstration of the mechanism responsible whether or not this particular pattern depends upon the existence of separate pathways therefore cannot be stated with certainty Perhaps the strongest evidence for dual pathways is the change in configuration of ventricular complexes reported by Moe, on the other hand nonuniform excitation of the component fibers in the bundle of His could produce the same result as could local dif- ferences in refractoriness of the peripheral Purkinje fibers Direct records from fibers in node His bundle and peripheral Purkinje system are required to provide a full explanation of these questions (see Chap 7)

Nodal Echoes Under certain conditions excitation originating in either atrium or ventricle enters the atrioventricular node and then returns to the chamber in which it originated This type of coupled extrasystole is often referred to as an echo Several authors have explained the appearance of echoes in terms of dual intercommunicat- ing pathways either in the node or extending from the node farther along the specialized atrioventricular conducting system (Moe et al , 1956 Rosenblueth 1958c) While it is possible that mechanisms

type of response which has been observed most frequently by a number of investigators In contrast, in a certain proportion of animals (dogs, goats turtles) Moe observed a different series of events (Fig 6 22*B*, *C*) At drive test intervals shorter than the functional refractory period of the atrioventricular conducting system there appeared either an abrupt (Fig 6 22*B*) or progressive (Fig 6-22*C*) increase in the delay between ventricular responses to drive and test stimuli This change in atrioventricular conduction time has been attributed to transmission through a slow path with a short functional refractory period (Moe et al, 1956) A similar abrupt change in conduction time has been observed during transmission of premature beats from ventricle to atrium (Moe et al, 1956, Rosenblueth 1958*b*) Also various experimental procedures have *been shown to alter the response pattern from that seen in Fig 6 22A* to that of either Fig 6 22*B* or *C* This phenomenon has been observed in heart lung preparations (Moe et al, 1956) and in in situ hearts following vagotomy excision of the upper thoracic sympathetic chains and adrenal ligation (Rosenblueth 1958*b*)

A considerable amount of indirect evidence has been presented in support of the existence of a dual conduction system Moe has shown that activity which presumably reaches the ventricle over the rapid system causes a different sequence of activation than that traversing the slower path Also atrial and ventricular echoes have been explained on the basis of intercommunications between the two pathways (Moe et al 1956, Rosenblueth 1958*c*) A detailed analysis of all available evidence supporting this hypothesis can be found in the original communications Anatomical identification of two separate pathways has not been provided It is uncertain whether or not the postulated dual conduction system is localized to the atrioventricular node or extends to the periphery of the ventricular Purkinje system (Moe et al, 1956) Moreover it should be emphasized that several aspects of the change in atrioventricular transmission time might be explained without assuming the participation of a dual system The usual observation (Fig 6 22*A*) is encountered even at the simple junction between ventricular muscle and Purkinje fibers during retrograde transmission of premature beats (see Fig 8-25) An abrupt change in latency of response in the Purkinje system with increasing prematurity of ventricular action potentials may also be observed (Fig 8 24) A change in atrioventricular transmis-

long, slow step which precedes the upstroke of the nodal action potential Similarly, the increments in atrioventricular delay are associated with nodal responses which arise later and later from the

A



B

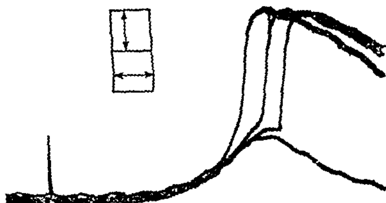


FIG 6-23 (*A*) Transmembrane potentials recorded from atrioventricular node (upper trace) and His bundle of rabbit heart showing the changes in nodal activity associated with the development of Wenckebach periodicity Note progressive increase in duration of the initial step of the nodal response, progressive increase in time interval between stimulation of the atrium (arrow) and the response of the bundle of His and the shortening of this interval after failure of nodal transmission (*B*) Unpublished record obtained by A Paes de Carvalho showing clearly the changes in activity of a single nodal fiber in rabbit heart during one Wenckebach period Calibrations represent 15 mv and 25 msec

step on the dropped beat the step is present, but no nodal action potential is observed

Similar changes in nodal activity can be seen more clearly in records obtained by A Paes de Carvalho (Fig 6-23*B*) It is apparent in these records that the primary change at the recording site is a decrease in the rate of rise and in the amplitude of the slow, steplike

potential change As a result of the decreased rate of rise and lower amplitude the local action potential arises progressively later Finally, when the step rises too slowly and fails to attain a sufficient magnitude, the local action potential does not appear

The records shown in Fig 6 23 suggest that the primary change in nodal activity causing delay and failure of transmission in these experiments occurs between the mid node and atrium since the responses which arise from the initial step of depolarization show a fairly consistent rising velocity and amplitude It is quite likely that the primary event is a progressive decrease in the amplitude of the action potentials near the atrionodal junction similar to that recorded during an abrupt increase in rate (see Fig 6-12) To date however, we have not succeeded in recording from the atrial margin of the node during Wenckebach periodicity and other changes in electrical activity near the atrial margin of the node cannot be excluded

7

THE PURKINJE FIBERS

The ventricular conducting system begins at the atrioventricular node and ramifies throughout the entire ventricle The extent and complexity of the ramification have been emphasized repeatedly (see Tawara 1906 Lewis, 1925 Baird and Robb 1950 Truex et al, 1954 Kugler and Parkin 1956) The vital role of this system should not be underestimated and an examination of the anatomical literature serves to impress one with the vast extent of the subendocardial Purkinje fiber network and with the degree of anastomosis between fine terminal Purkinje fibers and myocardial fibers

Fibers which answer the anatomical description of Purkinje fibers and which show action potentials similar to those seen in the bundle of His are found throughout that bundle, in free running strands (false tendons) in the ventricular cavity in an endocardial mesh in the chordae tendineae and even in the atrium Purkinje fibers have been demonstrated anatomically in almost all mammals and have been described in the turtle *Pseudemys elegans* (Robb 1953) Typical Purkinje fiber action potentials have been recorded in curious bundles occasionally found running free from the outer surface of the atrium to the outer surface of the ventricular base of the snapping-turtle heart (*Chelydra serpentina*) (Cranefield and Hoffman unpublished) It is thus reasonable when studying bird amphibian reptilian and mammalian hearts to assume that they contain a conduction system which is functionally important

THE PURKINJE FIBER ACTION POTENTIAL

The Typical Fiber

Configuration In any given species the action potential of the Purkinje fiber resembles the action potential of the ventricle to some extent (Fig 7 1) but there are certain marked differences The Purkinje fiber has a definite ' spike , that is phase 1 is prominent In addition the slope of phase 2 is low and the duration is longer than that of the ventricular fiber In general the Purkinje fiber action potential is the longest action potential found in any given heart In Purkinje fibers from the hearts of cat dog rat and snapping turtle we have usually found little or no diastolic depolarization under normal conditions Weidmann on the other hand working mostly with Purkinje fibers from the hearts of kids goats calves and sheep regularly notes pacemaker activity with a considerable degree of depolarization during phase 4 Purkinje fibers from all species will develop pacemaker activity under appropriate conditions

Magnitude Ample and reliable information is available on the magnitude of the resting and action potentials of Purkinje fibers The information is reliable because the fiber diameter is large Resting potentials are invariably found to be higher than 90 mv, and action potentials are over 120 mv (see Table 3 1)

The magnitude of these transmembrane potentials raises some interesting questions They are larger than the values reported for any other cardiac fiber types This does not necessarily mean that the other fibers actually have lower transmembrane potentials but may rather mean that only in Purkinje fibers have the transmembrane potentials been measured with full accuracy These fibers have the largest diameter of all cardiac fibers and also are the least contractile Both factors minimize damage to the cell membrane resulting from insertion of microelectrodes The effect of the insertion of a microelectrode has been studied in Purkinje fibers in a direct manner Draper and Weidmann (1951) employed one microelectrode to record resting and action potentials from a single fiber They then inserted another microelectrode less than 500 μ from the first, this second penetration of the membrane caused a drop of only 0 8 mv in the transmembrane potential recorded by the first Controls were carried out on the effect of cut ends of fiber bundles

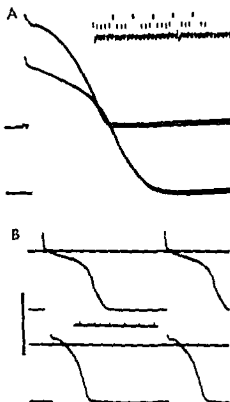


FIG 7 1 (*A*) Transmembrane action potentials recorded from papillary muscle (top trace) and attached Purkinje fibers (bottom trace) of the dog right ventricle Time calibration shows intervals of 10 and 50 msec (*B*) Transmembrane action potentials recorded from papillary muscle (bottom) and isolated false tendon (top) of the dog left ventricle Upper trace in both records is the line of zero potential Time calibration shows intervals of 100 and 500 msec Vertical bar at left of figure is a voltage calibration of 100 mv for *B*

junction potentials movement artifacts anesthetic agents, the use of Tyrode s solution, and the effect of frequency of contraction Few of these careful controls have been reported in studies of other fiber types

There is another factor which might operate to make the in vitro determinations of resting potential in Purkinje fibers nearer to their in vivo value than the measured resting potentials of smaller fibers would be This factor depends upon the size of the fiber Since the

ratio of surface area to cell volume is determined by the radius a larger fiber has a greater volume of cytoplasm for a given area of surface than has a smaller fiber If metabolism is impaired and if K^+ loss is proportional to cell area, then a large cell will lose K^+ at a slower rate relative to the total intracellular quantity and the intracellular concentration will fall more slowly than in a small cell Since metabolism is almost certainly impaired during in vitro studies, this argument weighs rather heavily in favor of the surmise that small cells are more likely to show reduced membrane resting potentials when studied in vitro

The argument from the surface area to volume ratio may, however, be used to support the view that smaller cells actually have a somewhat lower resting potential in vivo Thus the larger cell having a larger volume of metabolically active cytoplasm in relation to its surface area might maintain a higher resting potential even in vivo It has been found that the increase of oxygen metabolism associated with activity in axons is more nearly related to surface area than to volume and this observation has been interpreted to mean that the area does determine the amount of ionic movement and hence determines the need for metabolism, whereas the cytoplasmic volume is *an index of the metabolic capacity of the cell* (Connelly and Cranefield 1953)

Rate of Depolarization The maximum rate of depolarization in dog Purkinje tissue is 610 volts/sec and in kid Purkinje fibers 670 volts/sec (Draper and Weidmann 1951) All ordinary technical difficulties tend to reduce the measured rate of depolarization and it is possible that the true values approach 1 000 volts/sec In any case the rate of depolarization of Purkinje fibers is high and compares with the highest known rates of depolarization in any excitable tissue

Conduction Velocity Interest early attached to the conduction velocity in Purkinje fibers *because the realization that the* impulse spreads into the ventricles via the Purkinje system led some authors to attribute the atrioventricular delay to slow conduction in the Purkinje system In spite of the importance of the problem only a few direct attempts were made to measure conduction velocity in Purkinje fibers prior to the introduction of microelectrodes The first was that of Erlanger (1912) who overcame substantial technical difficulties and concluded that the conduction velocity was at least

0 75 m/sec in calf heart This result enabled him to state that the atrioventricular delay did not result solely from slow transmission through the conducting system (see Chap 6) In another study with external electrodes Curtis and Travis (1951) reported a value of 4 2 m/sec for isolated ox heart false tendons The same workers found that the conduction velocity varied linearly with temperature from about 1 2 m/sec at 13 C to 4 2 m/sec at 37°C Draper and Weidmann (1951) using intracellular electrodes found a conduction velocity of 2 0 m/sec in dog and 2 2 m/sec in kid heart A further study on dog Purkinje fibers (Trautwein Gottstein, and Federschmidt 1953) found a value of 2 5 m/sec Purkinje fibers thus show a higher conduction velocity than other types of cardiac cells (see also the last section of this chapter)

Duration Purkinje-fiber action potentials are longer than the action potentials recorded from other cell types in the same heart In dogs the Purkinje-fiber action potential may be 500 msec long This duration may be compared with that of the fiber of next longest duration the papillary muscle which is 250 to 300 msec (Hoffman and Suckling 1956) Purkinje fibers of the kid heart show action potentials of 400 msec duration (Draper and Weidmann 1951) and those of sheep heart about 500 msec (Weidmann 1956*b*) Duration is sensitive to rate temperature and ionic composition so all values are approximate Unpublished studies made in the authors' laboratory by A Paes de Carvalho suggest that the duration of Purkinje-fiber action potentials is different in different parts of the bundle of His and Purkinje-fiber network it seems probable that the fibers in the false tendons have action potentials of longer duration than in the bundle of His

Special Purkinje Fibers

The information given in the preceding section was in all cases obtained from fibers either of the bundle of His its branches or the false tendons ' (bundles of Purkinje fibers which run free through the ventricular cavity) Action potentials in these various sites do not show marked individual peculiarities There are however other sites which contain Purkinje fibers and they must be considered separately

The Atrioventricular Node The ventricular margin of the atrioventricular node contains fibers which are transitional in size and

action-potential shape between nodal fibers and His-bundle fibers The characteristics of these fibers are discussed in Chap 6, where they are referred to as "lower nodal fibers" They may also be regarded as Purkinje fibers

The Intramyocardial Purkinje Fibers Little information is available on the terminal branches of the conducting system Preliminary studies on rabbit ventricle (Paes de Carvalho et al, unpublished) suggest that the action potential of Purkinje fibers in this region may be shorter than in the free running branches

The Atrium Attention has been called to the presence of specialized conducting fibers in the atrium (Paes de Carvalho et al, 1959) These are discussed in Chap 3 It may be noted here that these fibers have action potentials rather similar to those of fibers of the false tendon

THE PURKINJE FIBER ELECTROGRAM

The action potential of an excised bundle of Purkinje fibers can be recorded with external electrodes and the electrogram registered in this way is typical of cardiac fibers in showing a QRS complex and a T wave (Curtis and Travis, 1951) It is also possible to record Purkinje-fiber action potentials in the heart by applying bipolar electrodes to the bundle of His or to one of its branches A few records of this sort are reported by Alanis et al, (1958) The method has been used in the authors' laboratory to examine the spread of excitation in the open dog heart maintained on a pump oxygenator (Fig 7 13) Results of those studies are described at the end of this chapter We usually record the action potential from the His bundle for purposes of time reference in studies of the atrioventricular node in vitro (see Chap 6)

EFFECT OF PHYSIOLOGICAL VARIABLES

Rate and Rhythm

The duration of the Purkinje-fiber action potential is very sensitive to rate (Fig 7 2) Only one reasonably systematic study of this effect has been reported (Trautwein and Dudel, 1954a) At a frequency of 400 per minute a duration of 100 msec was found while at frequencies of about 60 per minute the duration was about

500 msec Duration was found to bear an inverse and roughly linear relation to frequency over that range These figures show very forcibly the role of the shortening of the action potential in permitting a rate increase If rate induced shortening did not occur a fiber whose action potential duration at 60 per minute was 500 msec could be driven only at twice that rate since excitability is regained

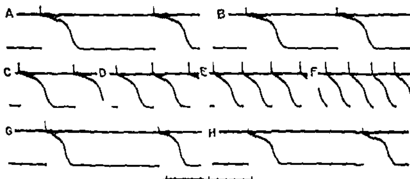


FIG 7 2 Transmembrane action potentials recorded from isolated dog Purkinje fibers showing changes in repolarization associated with changes in rate The driven cycle length is (*A*) 1 400 (*B*) 1 100 (*C*) 700 (*D*) 450 (*E*) 330 (*F*) 230 (*G*) and (*H*) 1 400 msec Note alternation in action potential duration in *F* and also the delayed return to control action potential duration in *G* and *H* Records in *F* *G* and *H* are separated by intervals of 30 sec Time calibration below record shows intervals of 100 and 500 msec

only near the end of phase 3 The rate-induced shortening of the action potential is such that a nearly sevenfold increase in rate may be obtained rather than a twofold increase

It should be noted that attempts to determine the refractory period are naturally influenced by the duration of the action potential and that therefore it is almost meaningless to determine the refractory period of the atrioventricular conduction system by determining the greatest frequency of impulses it can carry Increasingly rapid stimulation of the atrium will result in durational changes in every part of the heart Unless the durational changes and the corresponding changes in refractoriness are known it is very difficult to assign a site to the eventual atrioventricular block which develops Variations in rhythm also affect the duration of the Purkinje-fiber action potential A premature action potential is short and this shortness is observed not only near the site of

origin but also along the length of the fiber throughout which premature propagation occurs

Acetylcholine

Acetylcholine apparently is almost wholly without effect on Purkinje fibers (Fig 7-3) In particular reasonable concentrations

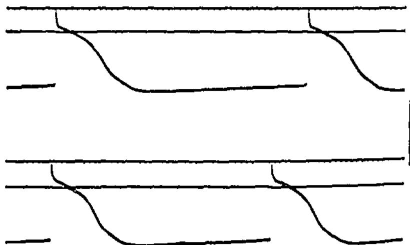


Fig 7 3 Transmembrane potentials recorded from isolated dog Purkinje fibers prior to (A) and after (B) addition of acetylcholine in a final concentration of 1 100 000 Top trace in each record shows time marks at intervals of 50 msec second trace from top shows line of zero potential Vertical bar at the right of the figure represents 100-mv calibration The decrease in action potential duration from A to B is due to the increase in spontaneous rate See text for discussion

of acetylcholine do not cause shortening of the action potential duration Very high concentrations do shorten the action potential slightly (Schmidt, 1958) An unexplained observation by the authors may point to an acetylcholine effect however When total atrioventricular block results from acetylcholine, we have found that the pacemaker for the "escaped" beat is not in the atrioventricular node but in the atrial portion of the bundle of His This portion of the bundle of His develops a spontaneous depolarization during phase 4 which it may not develop when the preparation is merely quiescent (see Fig 6-7) It is possible therefore that acetylcholine can induce pacemaker activity in certain Purkinje fibers

Epinephrine

The most obvious effect of epinephrine on Purkinje fibers is to increase pacemaker activity In fibers which show spontaneous depolarization during phase 4 the addition of epinephrine increases the slope of the depolarization and also lowers the threshold by increasing the threshold potential (Otsuka 1958) Both these effects increase the rate of spontaneous activity The change in threshold potential is the opposite of that seen in the sinoatrial node, in which the threshold potential decreases under the action of epinephrine

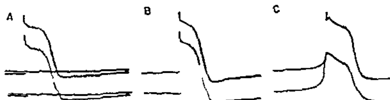


FIG 7-4 Transmembrane action potentials recorded from two sites in a single isolated dog Purkinje fiber showing the effects of l-epinephrine (*A*) Control (*B*) and (*C*) l-epinephrine 1 1 000 000 and 1 500 000 respectively Note increased rate increase in slow depolarization during phase 4, appearance of pacemaker activity at one recording site and associated changes in the upstroke of the action potential See text for discussion

or sympathetic stimulation (see Chap 5) In Purkinje-fiber preparations which show little or no spontaneous depolarization during phase 4 the addition of epinephrine produces it and leads to spontaneous firing (Fig 7-4) The rate of firing depends directly upon the amount of epinephrine added However other factors such as hypoxia or a decrease in the extracellular Ca^{++} concentration cause an appreciable increase in the sensitivity to epinephrine The authors' unpublished studies have shown that norepinephrine and epinephrine are equally potent in producing spontaneous activity in Purkinje fibers (Fig 7-4 7 5) We have also found that raising epinephrine concentration to a high level results in the development of multiple pacemakers in an excised Purkinje-fiber network and produces a picture closely resembling fibrillation A similar high concentration of norepinephrine has exactly the same effect (Fig 7 5) During development of this arrhythmia the transmembrane action potentials recorded from a single fiber initially show one or

more humplike depolarizations during the latter part of the plateau or the initial stages of phase 3 These depolarizations appear to prolong the plateau considerably and at a later stage of the arrhythmia the transmembrane action potential may show an initial fairly rapid upstroke and a plateau interrupted by numerous slow depolarizations of short duration (Fig 7-5) Simultaneous records from two

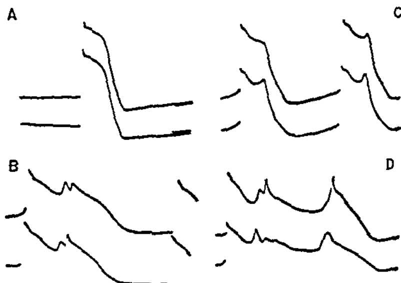


FIG 7-5 The effects of 1 norepinephrine on transmembrane potentials of isolated dog Purkinje fibers Same preparation as shown in Fig 7-4 *(A)* *Control* *(B)* norepinephrine 1 250 000 *(C)* and *(D)* norepinephrine 1 *100 000* Note evidence of repetitive activation at both recording sites and the associated prolongation of the action potential duration in *C* and *D* See text for discussion

or more fibers at this time show moderate to marked asynchrony of the small depolarizations and multifocal pacemaker activity

The effect of epinephrine on action potential duration in Purkinje fibers seems to be slight according to our own unpublished observations (see Fig 7-4) It must be realized that any increase in epinephrine to levels high enough to cause an increase in frequency of contraction will result in shortening of the action potential duration due to the accelerated rate No studies of the effect of rate on duration at various concentrations of epinephrine have been carried out

Ions

Sodium The most careful studies of the effect of Na^+ concentration on cardiac action potentials are those carried out on Purkinje fibers by Weidmann and his associates (Draper and Weidmann 1951, Weidmann 1955*a* Weidmann 1956*b*) Reduction of external Na^+ affects all phases of the action potential but the effect on

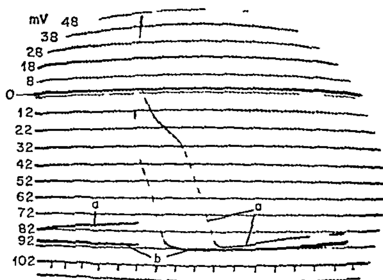


FIG 7 6 Effect of lowering the external Na^+ concentration The Purkinje-fiber action potential marked *a* is taken from a fiber exposed to a normal NaCl concentration that marked *b* from a fiber exposed to a solution containing 13 per cent of the usual NaCl concentration Time marks 100 msec (*Draper and Weidmann* 1951)

phase 0 will be considered first because of its importance in relation to the Hodgkin theory Draper and Weidmann (1951) found that reduction of external Na^+ concentration reduced the overshoot or membrane reversal without markedly changing the resting potential (Fig 7 6) Their results are in fair quantitative agreement with the predictions of the Hodgkin theory They also found that propagation failed if the external Na^+ concentration fell much below 20 per cent of normal and that reduction of Na^+ reduced the rate of depolarization seen in phase 0

Reduction of external Na^+ also affects phase 4 and phase 2 The steepness of the spontaneous depolarization during phase 4 is reduced by about 50 per cent when the Na^+ concentration is reduced to 25 per cent of normal (Weidmann, personal communication) This effect was discussed by Draper and Weidmann (1951), who point out that it accords with earlier observations that lowering of external Na^+ reduces the frequency of a spontaneous rhythm The steepness of phase 2 is, on the other hand, increased by a reduction in external Na^+ This results in a shortening of the action potential duration An increase in external Na^+ results in prolongation of the action potential It has been found that if LiCl is substituted for NaCl the action potential upstroke velocity and amplitude are maintained for a time, eventually both fall (Weidmann, personal communication) It is possible that Li^+ can enter the fiber but can not be extruded as easily as Na^+

Potassium The most obvious effect of an increase in extracellular K^+ concentration is a reduction in the resting potential Weidmann (1956*b*) has determined the resting potential of Purkinje fibers as a function of K^+ concentration (Fig 7-7) It can be seen that over a range of about 3 to 150 mM the resting potential is proportional to the logarithm of the external K^+ concentration This is in agreement with the prediction of the Nernst equation and is evidence in favor of regarding the resting potential as a K^+ concentration potential

The other effects of increased external K^+ concentration may result either directly from the K^+ concentration or indirectly from the reduction of resting potential Thus a reduction in action potential amplitude and in the steepness of phase 0 can be explained by the effect of a low membrane potential on the availability of Na^+ *carrier (see Chaps 2 and 9) Both effects* can be reversed by restoring the membrane potential to normal values with anodal current flow On the other hand an increase in external K^+ concentration also shortens the duration of the action potential chiefly by increasing the steepness of phase 2 The shortening is not fully reversed by anodal polarization, although phase 2 becomes somewhat more prominent and duration is increased (Weidmann, 1956*b*) It thus appears that external K^+ concentration exerts effects other than those secondary to depolarization If K^+ is reduced below 3 mM the resting potential declines sharply This observation is similar to that made on certain nerve fibers but has not been ade-

quately explained Interactions between K^+ concentration and Ca^{++} concentration are discussed in the section on Ca^{++} immediately below

Calcium It has been found that the shape and amplitude of the Purkinje-fiber action potential are relatively insensitive to changes

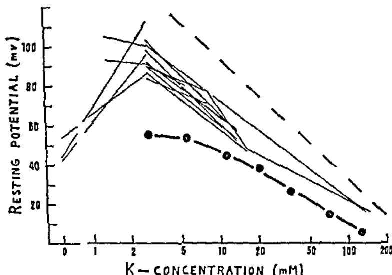


FIG 7 7 Resting potential as a function of external K^+ concentration Heavy line values from cat atrium (*From Burgen and Terroux* 1953*b*) Light lines sheep and calf Purkinje fibers Broken line theoretical curve corresponding to a 61 5-mv change in resting potential for a ten fold change in K^+ concentration (*After Weidmann* 1956*b*)

in Ca^{++} concentration Thus a sixteenfold change of external Ca^{++} from 0 65 to 10 4 mM (i e from 0 25 of the normal value to four times the normal value) produces little change in the resting potential or in the amplitude of the reversal (Weidmann 1955*b*) A greater reduction (to 10 per cent of normal) results in marked changes of a deteriorative nature The steepness of depolarization in phase 4 becomes marked the resting potential is reduced and the reversal seen in phase 0 is abolished (Hoffman and Suckling 1956) The action potentials in very low Ca^{++} become strikingly similar to those seen in a sinoatrial nodal pacemaker It is remarkable that if such a *reduction in* Ca^{++} *is made in the solution bathing a papillary* muscle with a Purkinje fiber attached to it perfectly normal action

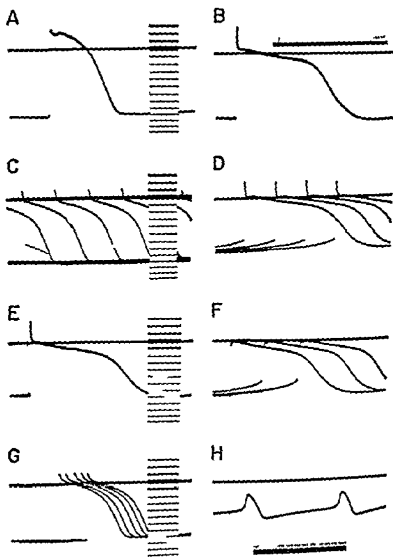


Fig 7 8 Transmembrane action potentials recorded from a Purkinje-fiber-papillary muscle preparation from the dog right ventricle (A) and (B) Control records from papillary muscle and Purkinje fiber respectively Ca^{++} concentration 2 7 mM (C) and (D) The effect of low-calcium concentration (Ca^{++}, 0 27 mM) on papillary muscle and Purkinje fiber Note spontaneous activity and appearance of slow depolarization during phase 4 of the Purkinje fiber records in D The response of the same Purkinje fiber to driving stimuli and to

potentials can be detected in the papillary muscle (Fig 7-8) at a time when the Purkinje fiber shows the deterioration described above moreover, the papillary muscle action potentials appear to be excited by propagation into the papillary muscle from the pacemaker of the deteriorated Purkinje fiber (Hoffman and Suckling 1956) At a slightly higher level of Ca^{++} (20 per cent of normal) considerable spontaneous depolarization during phase 4 and considerable reduction of the amplitude of phase 0 are observed If an extrasystole is induced in such a fiber the amplitude of phase 0 is normal It thus appears that the slow depolarization during phase 4 contributes (by inactivation) to the reduced amplitude of phase 0 Some fibers exposed to low Ca^{++} do not develop pacemaker activity but simply become partially depolarized and cannot be excited by cathodal stimulation If the transmembrane potential of such fibers is increased by anodal current flow anodal break excitation may give rise to an action potential when the current flow stops

A rather remarkable interaction between K^+ and Ca^{++} on the resting potential can be seen in Table 7 1 It will be seen that both

TABLE 7 1 THE EFFECT OF POTASSIUM AND CALCIUM ON THE RESTING POTENTIAL

Level of K^+	Ca^{++} normal	Effect of lowering Ca^{++}	Effect of raising Ca^{++}
Low K^+	Depolarization	Restores resting potential	Increases depolarization
Normal K^+	Normal resting potential	Normal resting potential	Normal resting potential
High K^+	Depolarization	Increases depolarization	Restores resting potential

marked reduction (to 0 48 mM or less) and increase of external K^+ cause depolarization If the Ca^{++} concentration is varied in the

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the intrinsic pacemaker is shown in *E* and *F* *G* and *H* show the effect of a further decrease in the calcium concentration (Ca^{++} 0 027 mM) on papillary muscle and Purkinje fiber respectively Voltage calibration in steps of 10 mv applies to each pair of records Top trace in each record is line of zero potential Time calibration in *B* applies to all records but *H* and shows 10- and 50-msec intervals Time calibration in *H* also shows intervals of 10 and 50 msec (*Hoffman and Suckling* 1956)

same direction as the K^+ concentration, the K^+ effects are minimized, whereas if the Ca^{++} concentration is varied in the opposite direction to the K^+ concentration, the K^+ effects are enhanced If the K^+ is normal a change in Ca^{++} does not affect the resting potential The results are in general accord with the idea of an antagonism between Ca^{++} and K^+ and are similar to those found in other excitable tissues (Shanes 1958) It has also been found that the increased rhythmicity seen in low Ca^{++} is enhanced by lowering K^+

The effect of Ca^{++} on the threshold of Purkinje fibers has been carefully studied It is known that the threshold to external stimuli changes when Ca^{++} is increased or decreased, but this could result from a change in membrane potential, membrane impedance, or the threshold potential In fact it has been found (Weidmann, 1955b) that a *fourfold* decrease or a *fourfold increase* in Ca^{++} is without effect on resting potential or membrane impedance The threshold change results from a shift in the threshold potential An increase in Ca^{++} decreases excitability by lowering the threshold potential, and a decrease in Ca^{++} increases excitability by increasing the threshold potential It must be remembered (see Chap 8) that threshold rises as threshold potential decreases provided that other factors remain constant a decrease in threshold potential means that a greater depolarization is needed to bring the membrane potential to the threshold potential

Weidmann (1955b) found that the slope of the spontaneous depolarization in phase 4 was constant under a *fourfold increase* or decrease in Ca^{++} concentration The frequency of spontaneous activity changed because of the change in threshold potential Thus an increase in Ca^{++} decreases the frequency of spontaneous activity because the depolarization seen in phase 4 must progress for a longer time before the threshold potential is reached It was also found that the rate of rise of phase 0 increased when Ca^{++} was raised This finding is discussed below in the section on current flow

Magnesium The only study of the effect of Mg^{++} concentration on Purkinje fibers (Hoffman and Suckling 1956) found results much like those long known for nerve If Ca^{++} is normal, the elevation or reduction of Mg^{++} produces very little effect, and if Ca^{++} is high the fiber is entirely insensitive to changes in Mg^{++} concentration No results are reported on the effect of Mg^{++} concentration when Ca^{++} is low

Temperature

Reduction of temperature from 40 to about 25°C causes a very small decrease in the resting potential of Purkinje fibers The decrease is roughly proportional to the absolute temperature and thus is in accord with the Nernst equation (Coraboeuf and Weidmann 1954 Trautwein Gottstein and Federschmidt 1953) Below about 25°C the resting potential falls somewhat more sharply to as low as

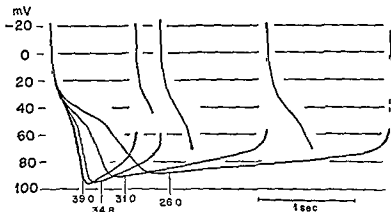


FIG 7 9 The effect of cooling on action potentials recorded from a spontaneous pacemaker in a Purkinje fiber Numbers below records indicate temperature in degrees centigrade (*Coraboeuf and Weidmann* 1954)

60 mv Since the drop in resting potential causes so many secondary changes the effect of temperature on the action potential is best examined at temperatures above 25 C

As would be expected cooling within the range from 40 to 25 C causes a great prolongation of the action potential and a reduction in the frequency of spontaneous activity if such activity is present The slowing of spontaneous activity results from a decrease in the slope of spontaneous depolarization during phase 4 The prolongation of the action potential similarly results to a considerable degree from a reduction in the slope of phase 2 All the electrical events of the action potential proceed more slowly when the temperature is lowered but the 'pacemaker phases phases 2 and 4 are the most sensitive to temperature change Coraboeuf and Weidmann (1954)

have measured the sensitivity to temperature of the various phases of the action potential, and their results can be seen in Fig 7 9 The numerical values for Q_{10} which they found from the same data are phase 0, −1 68, phase 1, −1 87, phase 2, −4 64, phase 3, −2 58, phase 4, −6 20 These values were constant over the range from 25 to 40°C It should be noted that a constant Q_{10} implies an exponential relation between temperature and steepness of the phase The great prolongation of the action potential with cooling may easily lead to artifactual introduction of rate effects If a heart or an in vitro preparation is driven at a fixed rate which is slow enough to cause no rate shortening at a high temperature, that rate may be fast enough to cause rate shortening at a lower temperature

The Effects of Current Flow

Two major aspects of the effect of current flow on Purkinje fibers are discussed elsewhere Excitation is discussed briefly below in this chapter and more extensively in Chap 8 and the evocation of regenerative and propagated repolarization by anodal stimuli is discussed in Chap 8

Certain systematic studies of the effect of resting potential on the action potential have been carried out with reference to the ionic theory of the action potential One finding of Hodgkin and his coworkers was that the availability of Na^+ carrier in the squid giant axon depends upon the resting potential Reduction of the resting potential leads to a reduction of the ability of the fiber to develop an increased permeability toward Na^+ and thus to a reduction in the steepness and amplitude of the upstroke of the action potential Qualitatively similar findings have been reported for Purkinje fibers (Weidmann, 1955*a*) The steepness of phase 0 depends upon the resting potential and may be varied by varying the resting potential If the resting potential is low, the steepness of phase 0 is low and may be increased by anodal polarization sufficient to increase the resting potential The general significance of these observations is discussed in Chap 9, as is the effect of various agents on the relation between the resting potential and the action potential Since depolarization will of itself reduce the amplitude and steepness of phase 0 no demonstration of any such effect by any other agent is necessarily of significance if that agent produces a primary depression of the resting potential

The effects of a number of such agents are in fact at least partially reversible by anodal polarization Thus cocaine reduces the resting potential the amplitude of phase 0, and the steepness of phase 0 (Weidmann, 1955b) anodal hyperpolarization sufficient to increase the resting potential from 81 to 114 mv returns the action potential towards normal A similar reversal of the effect of high K^+ (Weidmann 1956b) and of nonspecific exhaustion of the fiber (Weidmann, 1957 Coraboeuf, Zacouto Boistel and Distel 1954) can be obtained but hyperpolarization is not necessary, mere restoration of the resting potential to normal suffices It is a common observation in our laboratory that a fiber which has not been excited for some time may show a rather low resting potential and an abnormal action potential When regular stimuli are applied to such a fiber, the resting potential increases slightly after each action potential and each succeeding action potential improves in configuration and amplitude A similar effect has recently been noted in atrium (Trautwein and Dudel 1958b) It is possible that an increase in K^+ permeability secondary to activity is responsible for the improved resting potential It seems evident that the improvement in the action potential is secondary to the improvement in resting potential and vice versa The favorable interaction between the two no doubt finds its clinical counterpart in the fact that an apparently well perfused quiescent heart may be inexcitable but will respond well if two or three beats are somehow induced

It should be noted that the quantitative relationship between the level of the resting potential and the steepness of phase 0 of the action potential can be altered Thus cocaine (see above) does not act simply by depolarization It also alters the relation between upstroke velocity and resting potential so that a lower upstroke velocity is found at a given resting potential That the fundamental connection between the two is not lost is seen from the fact that hyperpolarization restores the upstroke velocity An opposite effect is seen with high Ca^{++} A normal upstroke velocity is seen at a normal resting potential when Ca^{++} is increased fourfold but the effect of depolarization on action potential upstroke velocity is reduced (Weidmann 1955b) The relationship between upstroke velocity and resting potential in normal fibers and in fibers treated with high Ca^{++} and cocaine is seen in Fig 7 10 Effects similar to those of cocaine are seen when the Purkinje fiber is treated with high con

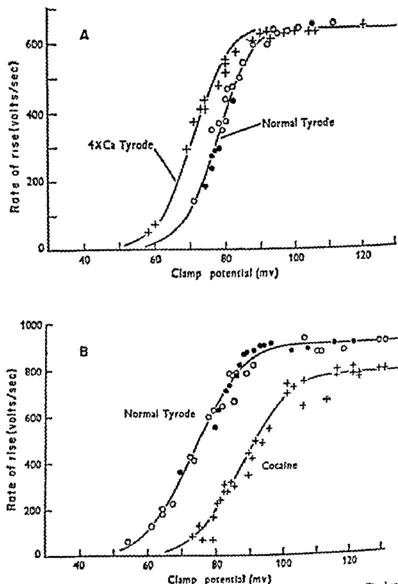


FIG 7 10 The relationship between the potential level of a clamped Purkinje fiber and the upstroke velocity of the action potential (A) The effect of calcium open circles normal Ca^{++} crosses $Ca^{++} \times 4$ solid circles after return to normal Ca^{++} (B) The effect of cocaine on the clamp potential and the upstroke velocity See text for discussion (*From Weidmann* 1955*b*)

centrations of procaine amide quinidine sulfate and diphenhydramine (Weidmann 1955b) Increase of external K^+, on the other hand produces no ' deleterious effect on the relation between resting potential and upstroke velocity The depression of the latter is in accord with the decrease in resting potential but is not exaggerated Full restoration of upstroke velocity results from mere restoration (not hyperpolarization) of the resting potential (Weidmann, 1956b)

Drugs

Digitalis A briefly reported study on the effect of digitalis on dog Purkinje fibers (Coraboeuf, de Lozé and Boistel 1953) found that a bath concentration of 1 40,000 causes an initial acceleration of rate followed by a slowing During the period of slow rate the thresh old is high and the action potential amplitude low Total arrest of activity supervenes During the initial period of increased rate the threshold is reduced (by elevation of the thre hold potential), and the steepness of phase 4 is increased The diminution of the action-potential amplitude appears before a marked fall in resting poten tial is seen The dose level required for these effects is clearly a toxic one

Quinidine It has been found that quinidine in very low (thera peutic) concentrations (3 to 6 μg/l) has two important effects on Purkinje fibers (Hoffman 1958) It reduces the slope during phase 4 both in spontaneously active fibers and in fibers in which depolariza tion during phase 4 is induced by epinephrine or low Ca^{++} (Fig 7 11) It prolongs the relative refractory period of both Purkinje fibers and papillary muscle fibers Quinidine exerts both these actions without depression of resting potential appreciable alteration of upstroke velocity or alteration of threshold The prolongation of the effective refractory period of the Purkinje fiber–papillary muscle junction occurs without marked prolongation of the action potential duration Procaine amide (30 to 60 μg/ml) was found to have exactly the same effect

In other studies (Coraboeuf Boistel, and Distel 1956, Weidmann 1955b) the dose of quinidine u ed was apparently toxic since it produced reduction of resting potential, diminished upstroke velocity and eventual loss of excitability The studies of Weidmann revealed the probable mechanism of the effect of quinidine on the refractory period Quinidine resembles cocaine in altering the rela

tionship between membrane potential and the ability of the cell to increase its Na^+ permeability Thus at a given degree of repolarization there is less recovery from inactivation in the quinidine-treated fiber than in the normal fiber

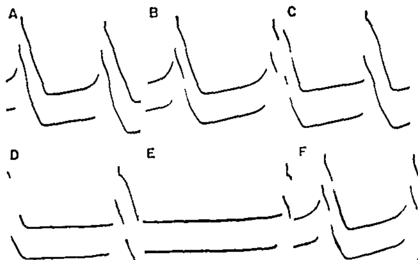


FIG 7 11 The effects of quinidine (6 μg/l) on spontaneous activity of isolated dog Purkinje fibers recorded at two different sites in the same preparation (*A*) Spontaneous activity induced by 1-epinephrine (*B*) through (*E*) effects of quinidine added to the same solution used for *A* (*F*) after washout of quinidine Note changes in the slope of phase 4 and the disappearance of the pacemaker type action potential recorded on the upper trace See text for discussion

Strophanthin The effect of toxic levels (5×10^{-6}) of strophanthin on Purkinje fibers has been studied (Dudel and Trautwein, 1958*a*) An early effect is the appearance of multiple extrasystoles in response to anodal break excitation in phase 3 The membrane resistance rises as judged by changes in the size of the electrotonic potential and the time constant This is interpreted as being the result of a decrease in K^+ permeability The action potential amplitude and the steepness of phase 0 fall off considerably at a time when the resting potential is still normal and a shift in the curve relating magnitude of the resting potential to steepness of phase 0 similar to that seen with cocaine is observed

Other agents

Anoxia The only detailed study of the effect of anoxia on the electrical response of Purkinje fibers (Trautwein, Gottstein and

Dudel, 1954) reports a shortening of the action potential duration and an eventual reduction of resting potential The shortening is initially disproportionate to the reduction of resting potential The steepness of phase 0 and the conduction velocity are not reduced immediately, but both eventually fall Anoxia eventually enhances spontaneous depolarization in phase 4 In the experiments of Trautwein et al it was necessary to drive the fibers at 150 per minute because fibers beating at a spontaneous rate of 50 per minute were extremely resistant to anoxia Upon the readmission of oxygen there is a rebound of recovery such that both resting potential and duration exceed control values

Carbon Dioxide and pH The effect of varying CO_2 concentration (in the gas mixture equilibrated with Tyrode s solution) from zero to 50 per cent has been reported (Coraboeuf and Boistel 1953) Increase of CO_2 from zero to 10 per cent results in a slowing of the spontaneous rate which is caused by a decreased slope of phase 4 When CO_2 is raised to 20 per cent a bigeminy results each normal beat being followed by a premature beat which appears to arise during the latter part of phase 3 A triple rhythm is also seen, in which the first premature beat is succeeded by another which arises in phase 3 of the first premature beat Increase of CO to 50 per cent results in a regular rhythm however the amplitude of the resting and action potentials fall and the fiber soon becomes inexcitable All the phenomena described are evoked in reverse order if the CO_2 concentration is again diminished The authors occasionally saw a "flutter in which a series of small subthreshold depolarizations occurred

Reduction of pH to 6 0 produces a curious response in Purkinje fibers (Coraboeuf Boistel and Distel 1955) Phase 3 is interrupted by a new depolarization which then usually gives way to repolarization Thus each action potential shows a hump of depolarization on its descending limb These humps may become new action potentials in which ca e a bigeminy is seen A small decrease in the concentration of $NaHCO_3$ in the Tyrode s solution markedly enhances spontaneous depolarization in Purkinje Fibers (Hoffman unpublished)

Stretch It has been found (Dudel and Trautwein 1954) that moderate stretch of Purkinje fibers yields little decrea e in resting potential Increasing the rest length even by 90 per cent causes a drop of only 15 mv while a stretch of 35 per cent causes no change

Considerable reduction in the steepness of phase 0 was seen with stretch above 30 per cent The most interesting observation was that even slight stretch (10 per cent) produced a tendency toward arrhythmia, associated with an increase in the slope of phase 4 Multiple foci of spontaneous activity were seen to develop in such fibers The eventual consequence was the development of multiple local foci none of which gave rise to propagated activity The significance of this observation may be appreciated if one considers the anatomical arrangement of the false tendons and small bundles of Purkinje fibers which run free in the cavity of the ventricle and are thus susceptible to stretch during filling

PASSIVE ELECTRICAL PROPERTIES

The only completely satisfactory analyses of the core-conductor properties of cardiac fibers are those which have been made on Purkinje fibers with intracellular electrodes The mean values obtained on kid false tendons (Weidmann, 1952) are as follows Fiber diameter 75 μ, λ, 1 9 mm, R_i, 105 ohm-cm, R_m, 1,940 ohm cm^2 τ_m 19 5 msec, C_m, 12 4 μf/cm^2 In a further study on calf and sheep hearts slightly different values were obtained, and temperature coefficients were determined The results follow R_i, 154 ohm cm, $Q_{10} = 1\ 48$ $R_m = 1\ 220$ ohm-cm^2 $Q_{10} = 1\ 49$, $C_m = 11\ 3$ $\mu f/cm^2$, $Q_{10} = 1\ 20$ (Coraboeuf and Weidmann 1954) The change in R_m during activity is of great interest but is difficult to determine Since the advent of microelectrodes, only one thorough study on one fiber type of one species has been reported That is the study made by Weidmann (1951) on kid Purkinje fibers The change in R_m was determined by subjecting the fiber to anodal pulses of long duration by means of one intracellular electrode and recording the change in transmembrane potential through another The variation in R_m is shown in Fig 7-12 It should be noted that R_m falls during phase 0 as the Hodgkin theory demands Great significance has been attached to the fact that R_m rises steadily during phase 1 and phase 2, and many theories of repolarization applicable to cardiac muscle have foundered on this observation The authors experiments have led us to question the observation (Cranefield and Hoffman, 1958b), but as yet the problem is unsettled This matter is discussed in Chaps 8 and 9

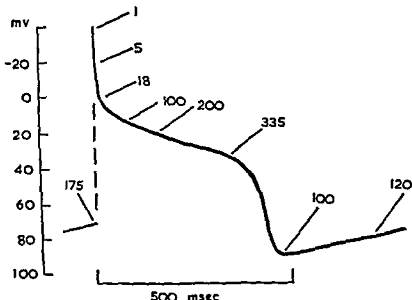


FIG 7 12 Changes in membrane resistance during electrical activity of kid Purkinje fiber The numbers attached to the curve indicate membrane resistance in relative units See text for discussion (*Weidmann* 1957*a*)

GENERAL REMARKS

Rhythmicity

The nature of spontaneous depolarization during phase 4 is discussed in Chap 5 Many of the results described there derive from, and apply to Purkinje fibers The principle problem concerning rhythmic activity in Purkinje fibers is the degree to which its presence can be regarded as normal It is found by some investigators as a regular in vitro phenomenon In most dog Purkinje fibers studied in our laboratory the transmembrane potential falls at most by a few millivolts during phase 4 Until it becomes possible to record in situ, the question is not likely to be resolved About the only statement which can be made is that a Purkinje fiber which shows a more rapid rate of spontaneous activity in vitro than is shown during idioventricular rhythm in the in situ heart from which it was taken is probably abnormal

The capacity to develop spontaneous activity e g, under the

influence of low Ca^{++} or epinephrine, is marked in Purkinje fibers and indeed it is so much more marked in them than in papillary muscle fibers that an important question arises Is all "spontaneous" activity of ventricular myocardial origin actually the result of spontaneous activity of the terminal twigs of the Purkinje system which pervade the myocardial wall? No definite answer can be given to this question, but the burden of evidence suggests that the answer is yes and that only very unusually, if ever, do true myocardial fibers develop spontaneous activity This view was advanced earlier by Lewis (1925, p 387) The existence of a varied complex of spontaneously active fibers in the atrium further suggests that the true myocardial fibers of the atrium also are not the source of atrial extrasystoles (see Chap 3) In general it seems that the more "embryonic" tissue, which is less specialized for contraction, is more rhythmic This implies that it is the contractile myocardial tissue which is "specialized" In this connection no one should fail to read Gaskell s brilliant chapter in Schafer's "Textbook of Physiology" (Gaskell 1900)

Duration

The fact that the Purkinje-fiber action potential is longer in duration than the action potential of myocardial fibers has many intriguing implications, one of which concerns the ventriculo-atrial interval in retrograde conduction The observation that the ventriculo-atrial interval is longer than the atrioventricular interval is made on intact hearts From direct studies of the atrioventricular node it seems that retrograde conduction through the atrioventricular node is nearly as rapid as is direct conduction and that the explanation for the greater ventriculo-atrial interval may in part lie elsewhere In fact in premature beats it may represent a lapse of time at the myocardial–Purkinje-fiber junction during retrograde conduction That a papillary muscle-fiber action potential can arrive at an attached Purkinje fiber while the Purkinje fiber is incompletely repolarized is known positively (Hoffman, Kao, and Suckling, 1957) When this happens, propagation into the His bundle occurs only after a latency It thus appears that one consequence of the long duration of the Purkinje fiber action potential is that the ventriculo-atrial interval may include a myocardial fiber–Purkinje-fiber junctional delay as well as a delay in the atrioventricular node, whereas

the atrioventricular interval depends only on conduction time and a delay in the atrioventricular node

The differing duration of the action potentials of the Purkinje fiber and the myocardial fiber also raise questions about the interpretation of studies of cardiac excitability It is known that if an electrical stimulus is applied through bipolar electrodes during the T wave excitation occurs at the anode (see Chap 8) and that a long stimulus response latency is observed It has not been conclusively demonstrated that excitation at this moment represents excitation of myocardial fibers and not Purkinje fibers It is by no means impossible that the anodal threshold of terminal branches of the Purkinje system is lower at some interval of the cardiac cycle than either the anodal or cathodal threshold of myocardial fibers

Finally the discrepancy in duration of action potential and refractory period of ventricular and Purkinje fibers may be of importance in the initiation and maintenance of fibrillation It has long been supposed that some discrepancy in the excitability of various cardiac cells must be involved in fibrillation, it may prove that the most important discrepancy is the one normally present at the junction of myocardium with Purkinje tissue

Contractility

Purkinje fibers are contractile and their contractility is greatly enhanced by epinephrine and high Ca^{++} It seems unlikely that normal or enhanced contraction of the Purkinje system plays any role in the mechanical activity of the heart or in the spread of excitation, but neither problem has been investigated

The U Wave

A variety of theories have been advanced to account for the appearance of the U wave in certain leads in the human electrocardiogram and for changes in the U wave observed during electrolyte disturbances and myocardial disease Two theories which have gained wide recognition are those of Lepeschkin (1957) and Schaefer (1951) Lepeschkin has proposed that the U wave results from afterpotentials in the ventricular transmembrane action potential This hypothesis appears unlikely in view of recent studies (Hoffman Cranefield Lepeschkin et al, 1959) in which afterpotentials recorded from perfused rabbit hearts were shown to bear no relationship

to the simultaneous record of transmembrane potential Schaefer has attempted to account for the U wave on the basis of certain mechanical factors (Schaefer, 1951 1957) We have made no direct tests of this latter hypothesis However, we believe that it is quite likely that the U wave of the electrocardiogram is merely the surface record of repolarization of the ventricular Purkinje system, that is to say that the U wave is the Purkinje-fiber T wave This conjecture is based upon knowledge of the electrical properties of Purkinje fibers which has recently been obtained by means of recording from single fibers Thus the action potential of Purkinje fibers is considerably longer than that of ventricular muscle and the phase of rapid repolarization of Purkinje fibers is coincident in time with the U wave It is also known that K+, which produces the most marked variations in the amplitude and timing of the U wave, has a more marked effect on phase 3 of Purkinje fibers than on phase 3 of ventricular muscle The observation that the U wave increases with stretch of the ventricles may readily be explained by noting that the Purkinje-fiber action potential is shortened when the fiber is stretched (Hoffman, unpublished observation) and that dilatation of the ventricle is likely to impose an unequal degree of stretch on various parts of the Purkinje system Thus the asynchrony of repolarization of different parts of the Purkinje system would result in a deflection of increased amplitude in surface leads It might be supposed that the mass of the Purkinje fibers is too small to generate a detectable deflection in records obtained from surface leads It may be pointed out that the U wave is low in amplitude and that it is most readily detected in precordial leads Moreover, the Purkinje fiber network is quite substantial, and the spread of activation through it is highly directional Repolarization of this system therefore produces potential differences which are less subject to "cancellation" than are those of ventricular muscle

THE IN SITU SPECIALIZED CONDUCTING SYSTEM

Most studies of the electrophysiology of Purkinje fibers have been carried out on isolated preparations in addition these preparations have for the most part consisted only of segments of the free-running Purkinje system contained in the false tendons Studies of the specialized conducting system in the intact in situ heart have usually

been indirect Recently a technique has been developed which permits direct recording from all parts of the in situ specialized conducting system of the dog heart (Stuckey et al, 1959) during total cardiopulmonary bypass Since total body perfusion and Langendorff perfusion of the heart are supplied by a pump oxygenator it it possible to open all four chambers of the heart and to record directly from the bundle of His the right and left bundle branches the free-running Purkinje fibers in the false tendons, and the subendocardial Purkinje-fiber network This technique has been employed rather extensively by the authors in collaboration with Jackson H Stuckey some of the unpublished results will be summarized at this point

Identification of Records

With the exception of the bundle of His and parts of the right and left bundle branches the ventricular conducting system of the dog heart can be seen clearly when the ventricles are opened The approximate location of the His bundle and bundle branches can be determined from well known anatomical landmarks If close bipolar electrodes of small diameter are applied to the endocardium at the appropriate sites propagated depolarization of the underlying part of the specialized conducting system is recorded as a sharp, rapid deflection in the local cardiac electrogram Simultaneous records of this sort obtained from the bundle of His from the left bundle branch and from the junction of the anterior false tendon with the anterior papillary muscle are shown in Fig 7-13 It can be seen that the His bundle electrogram follows local atrial activity by an interval comparable to the nodal delay and precedes all ventricular activity by 20 to 30 msec The electrogram from the left bundle branch also shows a sharp rapid deflection following shortly after the His-bundle electrogram and preceding electrical activity at the base of the septum by 30 msec During stimulation of the peripheral end of the cut right vagus nerve (Fig 7-13*B*) the interval between atrial activity and the His-bundle electrogram is increased in all other respects the sequence of activity is unaltered When, on the other hand stimulation of the ventricle causes retrograde excitation (Fig 7-13*D*) the sequence of excitation of papillary muscle left bundle branch and bundle of His is reversed and the interval between the His bundle electrogram and atrial activity is increased Tracings

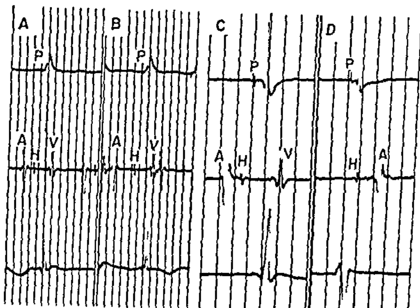


FIG 7 13 Records obtained by means of close bipolar electrodes from different locations on the endocardial surface of the in situ dog heart during total cardiopulmonary bypass by means of a pump oxygenator (*A*) and (*B*) Records from the left bundle branch (top trace) bundle of His (middle trace) and anterio papillary muscle (bottom trace) prior to (*A*) and during (*B*) vagal stimulation The deflections in the records are labeled as follows *P* Purkinje fibers *A* atrial muscle *H* fibers in the bundle of His *V* ventricular muscle Note increase in the interval between *A* and *H* during vagal stimulation (*C*) and (*D*) Records obtained from similar locations in a different experiment during normal (*C*) and retrograde (*D*) atrioventricular transmission See text for discussion

of this sort, in conjunction with anatomical studies serve to identify the records of each experiment

Electrograms of the Specialized Conducting System

The Bundle of His If close bipolar electrodes are located near the atrial end of the bundle of His the local electrogram is longer in duration and shows less sharp deflections than if the record is obtained from the distal end of the same structure In either location, however the electrogram shows little evidence of asynchronous activity in the His bundle Also of interest is the timing of the His bundle electrogram in relation to the complexes of the electrocardiogram and the *PR* interval It is apparent from records like the c

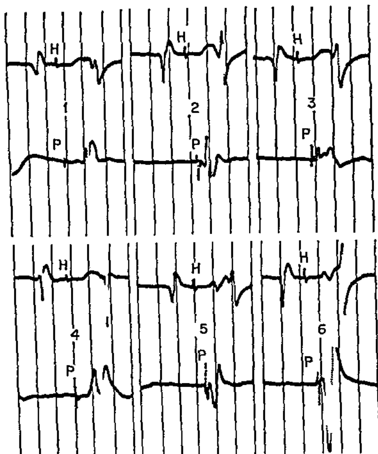


FIG 7 14 Bipolar electrograms recorded from the specialized conducting system at various locations in the in situ dog heart In all figures the top trace is recorded from electrodes located at a fixed point over the bundle of His The lower records in each figure are (1) right bundle branch (2) junction of moderator band with the right anterior papillary muscle (3) junction of Purkinje fibers with right ventricular free wall (4) left bundle branch (5) emergence of left anterior false tendon from the septum and (6) function of the left anterior false tendon with the anterior papillary muscle (*H*) action potential of His bundle (*P*) action potential of Purkinje fibers Changes in the configuration of the ventricular complex on the upper trace result largely from changes in the position of the heart during the experimental procedures Paper speed 200 mm/sec time lines 40 msec See text for discussion (*Hoffman Cranefield Stuckey et al* 19.9)

shown in Figs 7-13 and 7-14 that a large part of the electrocardiographically silent interval between the end of the P wave and beginning of the QRS complex is occupied by conduction in the His bundle, the bundle branches and the ventricular conducting system

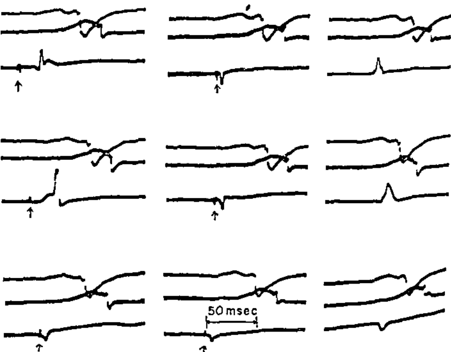


FIG 7 15 Electrograms recorded from the left septal surface of the in situ dog heart showing the electrical activity of various parts of the specialized conducting system The upper two traces are unipolar electrograms recorded from fixed electrodes attached to the epicardial surfaces of the left and right ventricles respectively The lower trace shows bipolar electrograms recorded through an exploring electrode located at various sites on the septal surface In each figure the initial small rapid deflection (arrows) represents the electrical activity of the subendocardial Purkinje fibers and the second deflection results from depolarization of the local ventricular muscle Note the differences in latency between activity of specialized fibers and adjacent ventricular muscle at the various recording sites See text for discussion (*Stuckey et al* 1958)

The Bundle Branches and False Tendons Electrograms recorded from the right and left bundle branches typically are shorter in duration than those of the His bundle and show more rapid deflections Polyphasic complexes are often recorded over the base of the

ft septal surface suggesting some asynchrony in the initial ranches of the left main bundle As can be seen in Figs 7 13 and 14 electrical activity of the left bundle branch precedes depolarization of the adjacent septal musculature by as much as 30 msec Records from the free-running false tendons are similar in all espects to those recorded from the bundle branches

The Subendocardial Purkinje Fibers A number of records have een obtained from the junction of the false tendons with the apillary muscle and from subendocardial Purkinje fibers at many oints on the right and left septal surfaces On the papillary muscles, ear the center of the left septal surface and just above the base of he anterior papillary muscle on the right septal surface, activity f the subendocardial Purkinje fibers precedes depolarization of djacent ventricular muscle by as little as 1 to 2 msec (Fig 7-14) The earliest activity of the septal muscle on the right and left sides s rarely separated by more than 1 to 2 msec At other locations the Purkinje-fiber electrogram may precede local ventricular activity by onsiderably longer intervals (Fig 7 15) In general records from ubendocardial Purkinje fibers are obtained much more frequently n the left septal surface this would be expected from results of natomical studies of the distribution of the specialized conducting system

Conduction Velocity in the Specialized Conducting System

Simultaneous records from the bundle of His from the right and left bundle branches from the false tendons, and from the junction of the false tendons with the papillary muscles (Fig 7-14) permit a direct calculation of conduction velocity in different parts of the specialized conducting system if accurate measurements of distance are made In the bundle of His of dog heart the conduction velocity is from 1 0 to 1 5 m/sec close to the atrioventricular node the velocity is still lower In the free-running Purkinje fibers of the false tendons the velocity reaches a value of 3 0 to 3 5 m/sec (Hoffman, Cranefield Stuckey et al 1959) Conduction velocity in both locations is the same during normal and retrograde propagation at normal heart rates Appreciable changes in velocity are not produced by vagal stimulation removal of the stellate ganglia, or administration of epinephrine or norepinephrine in doses causing the usual pressor response

Sites of Partial or Complete Conduction Block

Records of the transmembrane action potentials of single fibers of the His bundle and of single peripheral Purkinje fibers show that the action-potential duration increases progressively from the atrioventricular node to the false tendons (Fig 7-16) This finding suggests

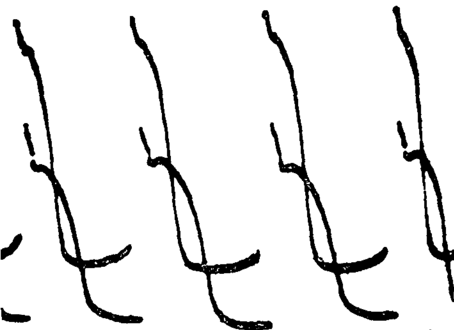


Fig 7 16 Transmembrane action potentials recorded simultaneously from single fibers in the bundle of His (upper trace) and ventricular Purkinje system (lower trace) of the rabbit heart Note marked difference in action potential duration at the two locations See text for discussion

that activity in the bundle of His might reach the peripheral Purkinje fibers prior to the end of phase 3 of the Purkinje-fiber action potential and that in addition to delay at the atrioventricular node local delay or block of conduction in the bundle branches or false tendons might occur When appropriately timed atrial extrasystoles are induced delayed conduction and block may be observed either in the peripheral Purkinje fibers the bundle branches (Fig 7 17), or between the His bundle and bundle branches (Fig 7-18) Also as might be expected from a comparison of the action potential dura

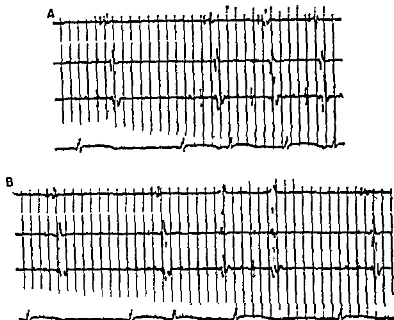


Fig 7 17 Bipolar electrograms recorded from several parts of the in situ specialized conducting system of the dog heart showing partial and complete block of appropriately timed atrial extrasystol s Top trace junction of anterior false tendon with anterior papillary muscle of left ventricle second trace emergence of left bundle branch from septum third trace record from over bundle of His bottom trace atrial electrogram In the two top traces the initial deflection indicates activity of the specialized conducting system and the second deflection indicates activity of the ventricular muscle in the third trace the initial complex indicates activity of atrial muscle the second complex indicates activity in the bundle of His and the final complex indicates activity of ventricular muscle In *A* note that an atrial extrasystole (third complex on bottom trace) reaches the bundle of His after moderate prolongation of nodal delay but then propagates with diminished velocity in the left bundle branch (note widened complex) and rea hes the papillary Purkinje junction after an additional delay In *B* note that an atrial extrasystole (third complex on bottom trace) occurring slightly earlier than in *A* propagates through the atrioventricular node and His bundle elicits only a small complex from the left bundle branch and fails to propagate to the peripheral Purkinje fibers excitation of the papillary muscle (top trace) and septal muscle (second trace) is thus delayed A similar block of the postextrasystolic beat is also apparent See text for discussion

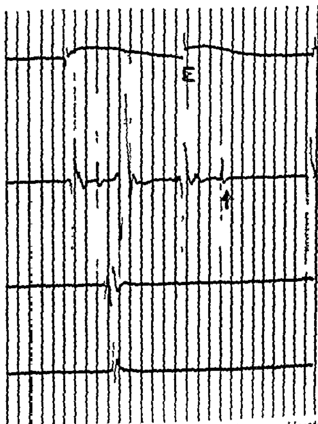


FIG 7 18 Simultaneous bipolar electrograms recorded at several locations in the in situ dog heart showing failure of transmission of an extrasystole from the bundle of His to the left bundle branch Top trace atrium second trace bundle of His third trace left bundle branch lower trace papillary muscle Note that the atrial extrasystole (*E*) results in a normal action potential in the bundle of His (arrow) but that there is complete failure of excitation of the Purkinje fibers in the bundle branch The absence of a ventricular complex on the His bundle record following the extrasystole shows that transmission to the ventricle failed completely and that concealed conduction of the atrial extrasystole is limited to the atrioventricular node and His bundle

tion of ventricular muscle and Purkinje fibers (see Fig 7 1) delay or local block of ventricular extrasystoles may occur at the peripheral junction of ventricular muscle with specialized fibers The importance of these observations in the understanding of certain abnormalities of cardiac conduction such as concealed conduction, echoes and reentrant rhythms is apparent, and further studies of this sort are in progress

8

EXCITABILITY

The study of cardiac excitability has revealed many new aspects of the electrophysiology of the heart in recent years A comprehensive review was published fairly recently (Brooks et al , 1955) but work has continued actively and there is much new material to be described in this chapter The determination of the threshold of the heart to electrical stimuli is only one facet of the study of responses of the cardiac cell membrane to current flow It will be seen that many responses other than 'excitation ' i e many responses other than the development of a propagated action potential, can be obtained by passing electrical current through the heart Particular theoretical interest attaches to the effects of anodal current flow during the action potential and much of this chapter deals with comparatively recent results in that area [1] The two major topics of this chapter are the effect of applied current and the effect of the natural electrical stimulus set up by the propagating action potential Unless otherwise stated all the results mentioned in this chapter were obtained on dog or cat hearts

Methods

The excitability of the heart is usually determined by applying electrical stimuli through surface electrodes Such electrodes must be attached to the myocardium in a way which does not injure the underlying tissue The presence or absence of injury can be ascer

[1] Many unpublished results which are presented in this chapter were obtained in the course of research supported in part by a grant to the authors from The American Heart Association

tained if electrograms are recorded periodically from the stimulating electrodes with a direct-coupled amplifier, the presence of injury is indicated by changes in the T wave, displacement of the ST segment, or the appearance of a monophasic complex It will be demonstrated below that the excitation of cardiac muscle can take place at both anode and cathode Therefore, whenever bipolar stimulation is employed, it is essential that both electrodes be placed on areas of myocardium that are activated simultaneously (Cranefield Hoffman and Siebens, 1957) The questions of size and relative position of stimulating and recording electrodes will be discussed below In studies of the recovery of excitability in cardiac muscle it is important to maintain a constant heart rate and to stimulate only occasionally because alterations in either frequency or rhythm have a marked effect on the duration of the action potential and therefore on the recovery of excitability The stimulus may excite intracardiac nerve fibers and therefore the possible liberation of chemical mediators must be taken into consideration This precaution seems most important when the heavily innervated atrium is the object of study

Single fiber Studies It is possible to stimulate a single cardiac fiber through an intracellular microelectrode This technique has several advantages, but it is more difficult to use than surface stimulation and as a result has been employed in only a limited number of studies Perhaps the most fruitful compromise is to stimulate with surface electrodes and to record the resulting change in transmembrane potential with one or more intracellular microelectrodes If care is taken to avoid distortion of the record of transmembrane potential by longitudinal current this technique permits a determination of the effects of stimulating current on the transmembrane potential and makes it possible to observe the development of local responses and propagated action potentials It should be noted that when an intracellular electrode is used to stimulate a reduction of transmembrane potential in the vicinity of that electrode occurs when the intracellular electrode is an anode

Stimulus Duration Classical strength duration curves for cardiac muscle have been obtained at various intervals during phases 3 and 4 of the cardiac cycle (Orias et al , 1950 Hoffman, Gorin, et al , 1951) The curves obtained with cathodal stimuli ranging in duration from 0 1 to 15 msec are essentially similar in shape but show much higher

thresholds for the shorter durations When anodal stimuli are employed, similar strength-duration curves are found The minimal durational requirement however, is longer for anodal than for cathodal stimuli Consequently the effects of anodal current are best studied with stimuli at least 10 m ec in duration

Latency The time which elapses between the stimulus and the re ponse is made up of the stimulus-excitation latency in the vicinity of the stimulating electrode and the conduction time from the stimu lus site to the recording site Both of these may vary with stimulus strength A threshold stimulus will excite more slowly than a supra-threshold stimulus Moreover a very strong stimulus can reduce the conduction latency by stimulating a large area surrounding the elec-trode and thereby giving rise to excitation farther away from the stimulating electrode and closer to the recording electrode

Threshold and the Threshold Potential The threshold of a tissue classically is defined as the minimum amount of energy required to elicit the typical response of that tissue In most cases for the sake of simplicity threshold is measured in terms of minimal electric current requirement It has been suggested that if the transmembrane potential is reduced to a critical level a propagated response will develop (Jenerick and Gerard 1953) This critical level is called the *threshold potential* A threshold stimulus in this sense is a stimulus just sufficient to reduce the transmembrane potential to that critical level (Fig 8-1*A*) There is a considerable body of evidence suggesting that excitation of cardiac muscle does in fact occur when the stimulus reduces the resting potential to a critical level (see Fig 8 1*B*) The concept of a threshold potential will be seen to contrast with the older idea that excitation depends upon reducing the mem brane potential by a critical amount A more detailed di cussion of the role of threshold potential resting potential and membrane resistance in determining excitability will be found in the chapter on the sinoatrial node (Chap 5)

Electrode Size Excitation results when a sufficiently large area of cell membrane is depolarized to or beyond the threshold potential Such a depolarization results from a flow of current through the membrane and the threshold requirement may be thought of in terms of a minimal current density through the membrane If a stimulus is applied with external electrodes only part of the current contributes to excitation namely the part which penetrates the

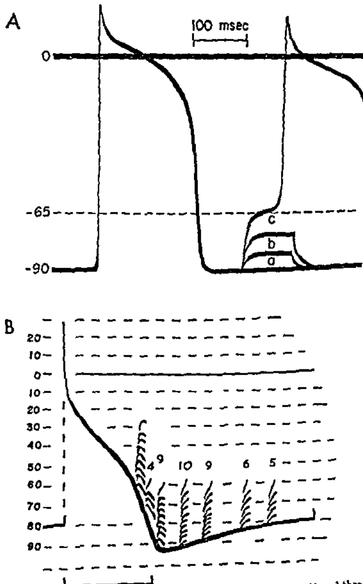


FIG 8-1 (A) Schematic record of the effect of subthreshold (a b) and threshold (c) depolarizing current pulses on the transmembrane potential of a single ventricular fiber Dashed line represents threshold potential See text for discussion (B) Tracing of the transmembrane potential of a single Purkinje fiber showing the relative strength of rectangular pulses of depolarizing current required to reach threshold at different times during phases 3 and 4 Numbers indicate the threshold current strength at each interval Horizontal dashed lines represent steps of 10 mv horizontal bar below figure represents 500 msec See text for discussion (Weidmann 1956b)

membrane Flow of current in extracellular paths does not contribute to excitation If one stimulating electrode is an intracellular microelectrode, all of the current flow penetrates the membrane In either circumstance, the maximum local current density through the membrane in the vicinity of the electrode depends upon the membrane resistance and the resistance of the intracellular and extracellular fluid

It had been supposed that the threshold of the heart to surface stimulation was independent of the size of the stimulating electrodes provided the electrodes were fairly large Subsequent experience showed that this was not so The threshold may be reduced by a factor of 100 by reducing the diameter of a surface electrode from 3 to 0 5 mm (Cranefield and Hoffman, unpublished) This fact has two important applications First it suggests that it is advantageous to use small electrodes when the supply of electrical energy is limited as it is in a chronically implanted artificial pacemaker Second true monopolar excitation may be obtained by using one small electrode and one large electrode (Cranefield Hoffman and Siebens 1957) If one electrode is 0 5 mm in diameter and the other is about 1 5 by 5 mm excitation will almost invariably occur at the small electrode irrespective of its polarity The advantage of this technique is that the location of the diffuse electrode is known, and should excitation arise there it can be detected by recording in that vicinity The cathodal and anodal excitabilities discussed in this chapter were determined with this technique If the 'indifferent' stimulating electrode is simply placed somewhere on the body the course of the return current is not known and excitation by the return current cannot readily be identified

CATHODAL STIMULATION

It has been shown in several studies that excitation can occur at either the cathode or anode during much of the cardiac cycle (Brooks et al 1955 van Dam et al , 1955 1956) Furthermore, it is known that if the excitability of the heart is tested with bipolar extracellular electrodes the response to threshold stimulation during phase 4 is initiated at the cathode whereas during phase 3 the threshold response occurs at the anode (Cranefield Hoffman and Siebens 1957 see also below) For this reason it is desirable to examine separately

the effects of anodal and cathodal stimulation This can be done if the area of one electrode is much greater than that of the other When an intracellular electrode is employed, a remote extracellular electrode is necessarily diffuse If surface electrodes are employed, special precautions, which are discussed in the section on methods are necessary to ensure subthreshold current densities at the diffuse electrode

Stimulation during Phase 4

When progressively stronger depolarizing stimuli are applied to fully polarized ('resting') cardiac muscle through either a surface or an intracellular electrode there is elicited first a local response and then, at a higher current strength a propagated action potential The local response which can be seen in Fig 8 2, is most easily

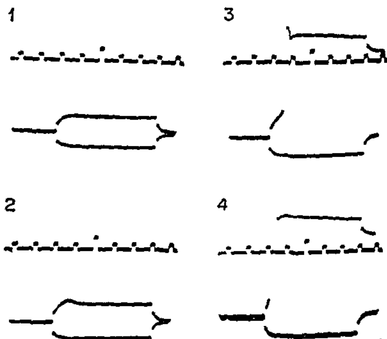


FIG 8-2 *Records of the transmembrane potential of a single ventricular* muscle fiber showing the effect of anodal and cathodal current pulses of equal strength applied to the fiber through an intracellular microelectrode Note subthreshold response in 2 and threshold response in 3 Upper trace shows time marks at intervals of 10 and 50 msec (*Unpublished record of Dr K Matsuda*)

recorded with an intracellular microelectrode The form of the propagated response as recorded with either surface or intracellular electrodes depends on the type of tissue under study The conduction velocity and the threshold-current requirement for depolarizing stimuli of fixed shape and duration are independent of the time of stimulation throughout phase 4 in a nonpacemaker cell

Stimulation during Phase 3

When depolarizing stimuli are applied during the terminal phase of repolarization (Fig 8-1*B*), the threshold current requirement is slightly less than it is during phase 4 This period of increased excitability presumably corresponds to the period of supernormality described by Adrian (1921) Hoff and Nahum (1938) and subsequent workers (Brooks et al 1955) The amplitude of the transmembrane action potential elicited at this time is often somewhat reduced It is reasonable to suppose that this supernormality results from the combination of a slightly reduced transmembrane potential with a threshold potential level which is the same as that found during phase 4 i e excitability is restored before the fiber is fully repolarized Studies of single fibers of isolated Purkinje tissue (Weidmann, 1955*b*) and of isolated papillary muscles (Hoffman and Cranefield unpublished) indicate that this interpretation is correct In healthy mammalian cardiac muscle this period is brief in duration and the increase in excitability is very slight It should be emphasized that the application of either threshold or suprathreshold stimuli during this interval results only in a single propagated response If the heart is stimulated earlier in the cycle when the membrane is less fully repolarized the muscle shows an elevated threshold and is said to be partially refractory The earlier in phase 3 the stimulus is applied the higher is the threshold until finally it is not possible to evoke a propagated impulse even with a very strong (1 000 times diastolic threshold) cathodal stimulus

If the electrical response of the heart is examined by recording with close bipolar electrodes at some distance from the stimulating cathode the propagated impulse resulting from a strong cathodal stimulus applied during phase 3 differs from an impulse evoked during diastole only in showing a longer stimulus-response latency If however the fibers near the cathode are examined with transmembrane recording, it is found that the response near the cathode is

quite different in phase 3 and phase 4 In phase 4, as shown above, the response to a threshold or suprathreshold stimulus is an action potential normal in shape and amplitude During phase 3, however, the fiber responds near the cathode with a depolarization which is slow in rise time, reduced in amplitude and short in duration (see Fig 8 22) The earlier an impulse arises, the more it departs from the normal shape and amplitude Rather careful studies with trans membrane recording (Kao and Hoffman 1958) suggest that the excitatory response evoked by cathodal stimulation during phase 3 may remain nearly stationary or may travel extremely slowly for less than 3 mm, in either case much of the latency results from the time required for adjacent tissue to recover, whereupon a nearly normal impulse propagates rapidly throughout the myocardial tissue more than a few millimeters away from the site of stimulation Such an impulse is normal in the sense that it presents a nearly normal shape, amplitude, and conduction velocity to an intracellular microelectrode located in nearby myocardial fibers It is abnormal in terms of the whole heart, since it is propagating through myocardium and not through the conduction system in the normal sequence

It is possible to obtain regenerative depolarization which does not result in propagation ("local response") by the use of subthreshold cathodal stimuli in phase 3 Nonpropagated responses have been detected by means of surface electrodes by Drury and Andrus (1924) and by Lueken and Schutz (1938) Drury inferred the existence of such responses from the observation that a stimulus which fell in the relative refractory period might prolong refractoriness even though it failed to evoke a propagated response The phenomenon observed by Schutz was different in nature In hearts subjected to low temperature or previous treatment with hypotonic sodium chloride solution he found that refractoriness outlasts repolarization During the time when the tissue was still refractory and after the monophasic action potential had returned to the resting level, stimulation evoked nonpropagated responses which were graded both in amplitude and duration (Schutz, 1936)

Stimulation during Phases 2 and 3

It is not possible to evoke a new propagated action potential by cathodal stimulation of the heart during phase 0 phase 1, phase 2

or during the first half of phase 3 However, the response of the brane is not wholly passive If sufficiently long and strong cat pulses are used, (Cranefield and Hoffman, 1958b) a prolongati the action potential may result (Fig 8-3) There is some

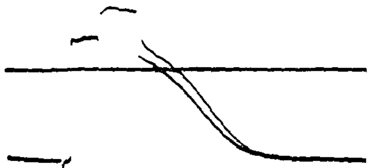


Fig 8-3 Transmembrane potential of a single fiber of cat ventricle show change in membrane potential resulting from a rectangular pulse of depol current of long duration applied during phase 3 Horizontal line represen potential

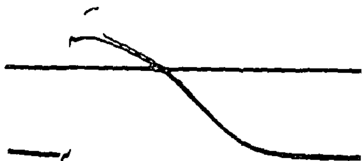


Fig 8-4 Record similar to that shown in Fig 8-3 showing the effect of of shorter duration

response even during a weaker and shorter stimulus since the membrane potential during the stimulus is found to be dis

The Relationship between Repolarization and the Recovery of the Cathodal Excitability

It will be seen below that anodal stimuli may excite even during phase 2, cathodal stimuli on the other hand, can excite only during phase 4 and the terminal part of phase 3 It is important to note that under certain conditions full restoration of excitability does not occur at the same moment as full repolarization Low temperature certain drugs and certain metabolic inhibitors appear to create a situation in which relative refractoriness outlasts repolarization Examples of such effects are to be found in Chaps 4 and 7 Such effects are very interesting and may moreover be of great significance in explaining the action of drugs The majority of studies on the action of drugs have been concerned with changes in the shape of the action potential It is often found that a disturbance in the relationship between repolarization and the recovery of excitability occurs at lower levels of drug concentration than do changes in the shape of the action potential It therefore seems most desirable to include studies of excitability in all studies of the effects of drugs and metabolic inhibitors on the electrical activity of the heart

ANODAL STIMULATION

The effect of anodal current is to hyperpolarize the resting membrane or to move the active membrane toward the resting state, i e, at any time during the cycle anodal current increases the degree to which the inside of the cell is negative with respect to the outside Since activity is associated with depolarization, excitation would not in general be expected to arise during the passage of hyperpolarizing current In fact, excitation at the anode usually arises after the end of the stimulus and is thus break excitation

Stimulation during Phase 4

It is possible to excite ventricular myocardium with anodal stimuli at any time during phase 4 The threshold is two to three times as great as the threshold to cathodal stimulation and it is reasonably constant throughout phase 4 Excitation always occurs after the cessation of the flow of anodal current (anodal opening excitation anodal break excitation) provided the stimuli are of reasonable

duration i e up to 20 msec Apparently excitation may arise at the anode during an anodal stimulus which lasts 30 msec or longer (Cranefield and Hoffman, unpublished) Anodal excitation in phase 4 has been demonstrated in atrial and ventricular tissue with external electrodes (Brooks et al , 1955, Cranefield Hoffman, and Siebens 1957) and in single fibers of the ventricle (Hoffman, 1959) and of the Purkinje system (Fig 8-21)

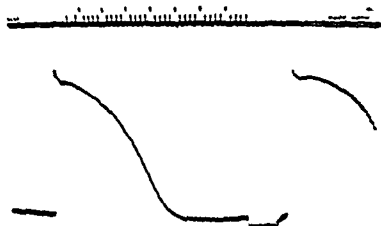


Fig 8-5 Transmembrane action potentials recorded from a single fiber of dog papillary muscle showing one driven response followed by an extrasystole initiated on the break of an anodal (hyperpolarizing) current pulse applied during phase 4 Time marks on upper trace show intervals of 10 and 50 msec see text for discussion

If the transmembrane potential is examined near the site of application of an anodal stimulus of short duration it is seen (Fig 8 5) that the fiber is hyperpolarized during the stimulus and that after the stimulus ends the transmembrane potential not only returns to the resting potential but continues beyond it in the direction of depolarization A slow foot of depolarization breaks into a full action potential However during phase 4 it is difficult to set the anodal stimulus strength to produce a local break response rather than a propagated action potential

Discussion Anodal break excitation has been held by some authors to be abnormal and not demonstrable in normal heart (Schaefer, 1942) It has been suggested that because of the complexity of cur-

rent flow in the intact heart, anodal excitation might be apparent only, the result of a "virtual cathode" The demonstration of simultaneous anodal and cathodal excitation in a well oxygenated in situ dog heart (Cranefield, Hoffman and Siebens, 1957) seems to rule out both these objections, particularly since the anodal threshold may be only double the cathodal threshold It has also been possible to demonstrate anodal excitation in single fibers both of the ventricle and of the Purkinje system (see above, Fig 8-21) These fibers appear normal and in particular do not have a diminished resting potential It is hard to suppose that excitation in the immediate vicinity of an internal microelectrode could arise at a "virtual cathode" It thus seems reasonable to assume that anodal excitability is a characteristic of normal cardiac cells

The mechanism of anodal excitation remains obscure The anodally excited single cell shows a depolarization which results in excitation The "discharge" of the anelectrotonus after the end of the stimulus provides a current flow of an excitatory direction Cardiac fibers in all probability show partial inactivation even at their normal resting polarization, so that hyperpolarization would be expected to increase the amount of available Na^+ carrier (Weidmann 1955a) It is not clear that this would be a sufficient explanation of anodal excitation but such an increase in available carrier would enhance excitability

Stimulation during Phase 3

Propagated Impulses It is possible to obtain propagated impulses during much of phase 3 in response to anodal stimuli of adequate strength and duration If an anodal stimulus is applied progressively earlier during phase 3 the threshold first rises slowly, then falls and then rises sharply (Fig 8-6) The initial increase in threshold takes place during the terminal portion of phase 3 and overlaps parts of the cathodal supernormal period and relative refractory period The subsequent drop in threshold seen during the middle third of phase 3 is so marked that the anodal threshold at this time is actually lower than the anodal threshold during phase 4 During this drop the anodal threshold is much lower than the cathodal threshold and remains lower throughout the early part of phase 3 (Fig 8-6) All propagated responses elicited during phase 3 show a conduction latency which is greater the earlier the stimulus is applied and which

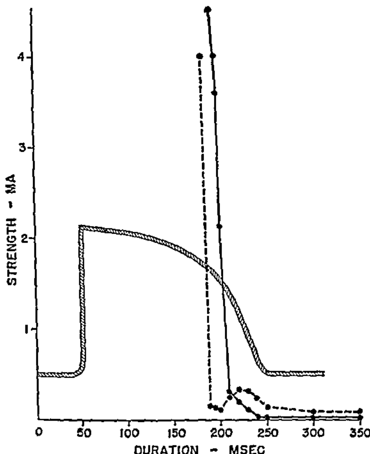


FIG 8-6 Strength interval curve of dog ventricle Thresholds obtained with a punctuate electrode and a diffuse electrode Solid line thresholds when the punctuate electrode was a cathode Broken line thresholds when the same punctuate electrode was the anode A monophasic action potential recorded from the site of the punctate electrode is superimposed Time zero is the drive time (*Cranefield Hoffman and Siebens* 1957)

is always greater than that seen after full recovery Records of the transmembrane potential obtained in the vicinity of the anode with an intracellular microelectrode show accelerated repolarization during the current flow and break excitation at the end of the current flow Both the amplitude and rising velocity of the response are

greater than those of a response evoked by a threshold cathodal stimulus applied at the same time during phase 3

Local Response Subthreshold anodal stimuli applied at any time during phase 3 are followed by local responses which are graded in accord with the strength of the stimulus With a threshold anodal stimulus the action potential is seen to arise from the peak of the local response Following a subthreshold anodal stimulus a short, weak, cathodal pulse applied at the peak of the postanodal local response gives rise to a full sized action potential The postanodal local response probably corresponds to the local responses elicited by cathodal stimuli from a membrane which is more fully polarized

The term *local response* generally is not applied to an action potential which is almost normal in amplitude and shape However, since the repolarization caused by anodal current may be localized to an area quite close to the electrode the break response may remain a phenomenon which is local even though it shows full amplitude and duration Under certain conditions large cathodal responses during phase 3 also are local in nature (Kao and Hoffman 1958) The possibilities for propagation of anodal break responses during phase 3 thus depend on the recovery of excitability in areas of membrane adjacent to the stimulating electrode

The No-response Phenomenon One of the most remarkable results of the studies of cardiac excitability by Brooks and his coworkers was the discovery of the no response phenomenon (Brooks et al, 1950 1955) This phenomenon was first observed in the atrial and ventricular myocardium of the intact heart It can be obtained with anodal stimulation during phase 3 At the time of the anodal supernormality described above it is found that an increase of stimulus strength above threshold may result in loss of excitation This failure of a stronger or longer stimulus to excite at the interval in the cardiac cycle when a weaker or shorter stimulus will produce a propagated action potential is the no-response phenomenon

The no response phenomenon was originally observed with bipolar stimulation but is more easily studied with monopolar anodal stimulation The use of this technique (Cranefield, Hoffman and Siebens, 1957) has shown that the no-response phenomenon is exclusively associated with anodal stimulation i e it is only anodal stimulation which may fail to be effective following an increase in strength or duration The period during which the no-response phe-

nomenon can be evoked is rather long (20 msec) and even extends into that part of phase 3 in which the anodal threshold is higher than the cathode threshold Apparently excitation at the anode does not reappear even if the stimulus strength is increased far beyond the no-response level Thus it is found that the range of strength and duration which will yield anodal excitation during the early part of phase 3 is exceptionally narrow, and it is very easy for a stimulus to be either subthreshold or above the no-response threshold (Fig 8-7)

Extrasystoles and Fibrillation It is known that the "dip in the anodal excitability curve corresponds to the vulnerable period during which the heart is prone to fibrillate in response to a single supra threshold electrical stimulus (Brooks et al, 1955) Fairly careful studies have shown that it is always possible to obtain single propagated action potentials (of unknown site of origin) by the use of extremely strong stimuli during the period when the no-response phenomenon is the usual response to anodal stimuli (Cranefield, Hoffman and Siebens, 1957) The period of vulnerability to fibrillation does not therefore appear to be coincident with the period when the no response phenomenon is most readily elicited (i e the early part of phase 3) The vulnerable period appears later, in the latter part of phase 3 and during maximum anodal supernormality In general it appears that fibrillation evoked by a single bipolar stimulus during phase 3 is associated with the development of a series of extrasystoles originating in the vicinity of the anode However such a series of anodal extrasystoles may occur without provoking fibrillation the onset of which is always signaled by the appearance of an 'extraneous' impulse which follows the burst of anodal extrasystoles and which does not originate in the near vicinity of either anode or cathode (Brooks Cranefield Hoffman and Siebens unpublished) If the flow of anodal current is sufficiently diffuse it is so difficult to evoke fibrillation that it seems safe to assume that fibrillation evoked by electrical stimulation depends upon anodal excitation

Discussion In the absence of an adequate explanation of anodal excitation in phase 4 it is difficult to surmise why there should be a "dip" in the anodal threshold in phase 3 It seems obvious that the repolarization which is in progress is accelerated by the anodal stimulus It is possible that the anodal supernormality seen in phase 3 results from a combination of anodal break excitation with

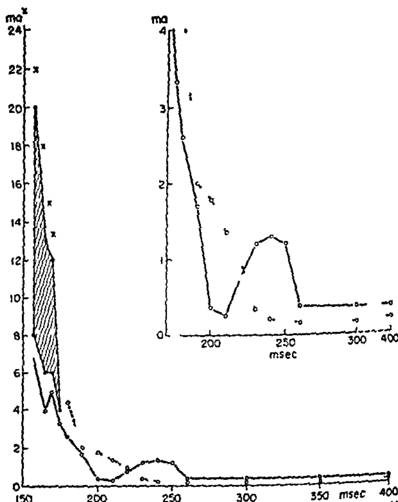


FIG 8-7 Strength interval curve for dog ventricle Solid line anodal thresholds broken line cathodal thresholds Strength and interval in milliamperes and milliseconds Inset dip portion of the curve on an expanded time and strength scale Cross hatched area an area of no response Y excitation of unknown origin of supra no response threshold (*Cranefield Hoffman and Siebens 1957*)

the flow of depolarizing current from nearby depolarized areas The contribution from nearby depolarized areas would of course be absent during phase 4 It should further be pointed out that fairly strong anodal pulses applied during the period of anodal superror mality effect greater changes in the transmembrane potential than

do pul es of the same strength applied at any other time in the cardiac cycle (Hoffman and Cranefield unpublished) It might therefore be expected that the stimulating efficacy of the anode would be increased since the postanodal discharge of the hyperpolarization (the classical "counter current") might be greater A purely speculative explanation of the no-response phenomenon has been developed along similar lines Increasing the strength of the anodal timulus might increa e the area of repolarization so much that the excitatory flow from depolarized areas would be abolished (Cranefield Hoffman and Siebens, 1957) This argument has been strengthened by the subsequent demonstration of propagated repolarization

Similarly, no satisfactory explanation is available for the ability of strong anodal stimuli to provoke multiple extrasystoles and even fibrillation during phase 3 An analogous phenomenon in nerve Ritter s opening tetanus de cribed in 1805, also remains without satis factory explanation It is generally stated however that Ritter's opening tetanus can only be elicited from partially depolarized nerves It is therefore interesting to note that even very strong anodal stimuli applied during phase 4 elicit only a single response whereas the first of the multiple responses in phase 3 begins at a time when the fiber is partially depolarized

Stimulation during Phase 2

Regenerative Repolarization During all of phase 2 and part of phase 3 the flow of anodal current not only accelerates repolarization but does so in a fashion which has been described as regenerative When anodal and cathodal pulses of equal strength and duration are applied to the ventricular myocardium it is seen (Fig 8 8) that greater effects result from the anodal pulse Moreover it can be seen (Fig 8-9) that if the strength of an anodal pulse of fixed dura tion is increased in equal increments the repolarization of the fiber in the vicinity of the anode does not increase in equal steps but shows a nonlinear increase as the stimulus strength increases This effect has been demonstrated in Purkinje fibers (Weidmann 1951) and in dog and cat papillary muscle (Cranefield and Hoffman 1958b)

All or None Repolarization A direct though not necessary consequence of the existence of regenerative repolarization in response to

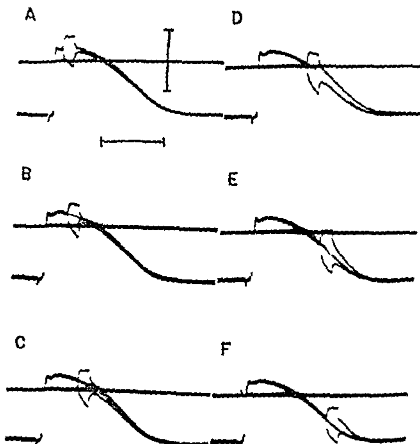


Fig 8-8 The effect on membrane potential of repolarizing and depolarizing pulses of 20 msec duration and constant strength applied at different intervals during the action potential Time calibration 100 msec voltage calibration 100 mv (*Cranefield and Hoffman* 1958b)

anodal stimuli applied during phases 2 and 3 is the existence of all-or none repolarization If the anodal stimulus is sufficiently long and sufficiently strong the action potential does not resume its original course after the cessation of the pulse instead the fiber remains fully repolarized (Fig 8-10) All-or none repolarization of Purkinje fibers was discovered by Weidmann (1951) and subsequently shown to exist in cat and dog ventricular fibers by Cranefield and Hoffman (1958b) A similar phenomenon has also been demonstrated in nerve

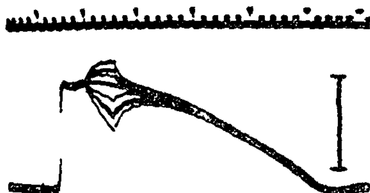


Fig 8-9 The effect of depolarizing and repolarizing pulses of 30 msec duration on the action potential of a single fiber of papillary muscle Time calibration 10 msec and 50 msec voltage calibration 100 mv (*Cranefield and Hoffman* 1958b)



Fig 8-10 Transmembrane potential of a single Purkinje fiber of dog heart showing one uninterrupted action potential and superimposed the effect of a pulse of hyperpolarizing current applied to the same fiber through a second microelectrode causing sustained early repolarization

fibers (Tasaki, 1956, Tasaki and Hagiwara, 1957) and in a cobalt wire model (Tasaki, 1957)

Propagated Repolarization The existence of regenerative repolarization and of all or none repolarization strongly suggests the possibility of propagated repolarization a phenomenon in fact demonstrated by Weidmann (1951) in Purkinje fibers The discovery of

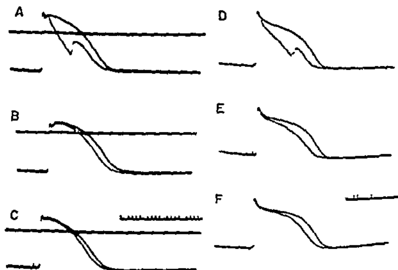


Fig 8-11 Records showing the effect on the membrane potential of a single fiber of papillary muscle of repolarizing pulses sufficiently strong to initiate propagated repolarization All records obtained by differential recording between one microelectrode inside the membrane and another located just outside the same area of membrane (*A*) (*B*) and (*C*) Normal Tyrode solution record taken 0 5 mm (*A*) 3 0 mm (*B*) and 5 5 mm (*C*) from the polarizing electrode (*D*) (*E*) and (*F*) Solution containing 25 per cent of the normal amount of Ca^{++} Records taken 0 5 mm (*A*) 2 0 mm (*B*) and 4 0 mm (*C*) from the polarizing electrode Time marks in *C* and *F* show intervals of 10 and 50 msec See text for discussion (*After Cranefield and Hoffman 1958b*)

propagated repolarization was foreshadowed by the largely neglected work on propagated relaxation carried out by Biedermann (1895) It has also been possible to demonstrate the existence of propagated repolarization in cat and dog ventricular fibers (Cranefield and Hoffman 1958b)

Propagation of repolarization is shown in Fig 8-11 It will be noted that when all-or none repolarization is achieved in the vicinity of the anode, shortening of the action potential is observed at a distance The mechanism of propagated repolarization must be

basically similar to the mechanism of propagated depolarization and depend on the flow of repolarizing current between a repolarized and a depolarized area A repolarized area is anodal with respect to a depolarized area The flow of current between a depolarized and repolarized area at one and the same time tends to excite the repolar ized area and to repolarize the depolarized area It would be ex pected therefore that under certain conditions the anodally repolar ized area might become reexcited instead of the adjoining depolarized

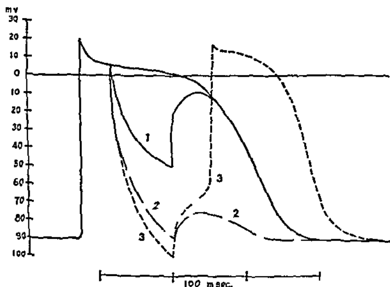


Fig 8-12 Diagrammatic representation of the effects of anodal current pulses of three different strengths applied during phase 2 See text for di cussion

areas becoming repolarized This is found to be true and is discussed in the following section

Break Reexcitation It is found in cat and dog papillary muscle that the following sequence of events can be obtained by progressively increasing the strength of an anodal stimulus applied during phase 2 (1) the action potential resumes its normal course after the end of the anodal pulse (Fig 8 12 1) (2) the fiber remains repolar ized (Fig 8-12 2) (3) the fiber fully repolarized exhibits break reexcitation at the end of the anodal pulse (Fig 8 12 3) Break reexcitation which presumably results from a combination of anodal break excitation and the excitatory flow of current from adjoining

depolarized areas, is the usual response of normal ventricular muscle to strong anodal stimuli It is, therefore, difficult to demonstrate propagation of repolarization in this preparation It has been found (Cranefield and Hoffman, 1958b) that reduction of the external Ca^{++} concentration makes it more difficult to obtain break reexcitation and therefore easier to obtain propagated repolarization It also has been found in the squid giant axon that reduction of the Ca^{++} concentration in the extracellular fluid decreases the rate at which inactivation is removed under an anode (Frankenhaeuser and Hodgkin 1957) This means that repolarization of an excited fiber restores the excitability more slowly than under normal conditions In general it seems likely that sustained repolarization will predominate over break reexcitation whenever refractoriness lasts longer than repolarization Both low temperature and low Ca^{++} have this effect

The separation of the threshold for sustained repolarization and break excitation makes it easier to study break reexcitation in such a preparation It can be seen (Fig 8 13) that a sequential increase in the strength of a series of anodal stimuli yields progressively higher levels of membrane potential and eventuates in a sustained and complete repolarization Further increase of anodal stimulus strength beyond this point results in break reexcitation which is graded in amplitude and duration both increase when the strength of the stimulus is increased With a very strong anodal stimulus a new, full action potential can be elicited at almost any time during phase 2 However, this action potential usually does not propagate for any appreciable distance

Discussion The observation that anodal current flow initiates regenerative repolarization in phase 2 raised many interesting problems which have been discussed in some detail (Cranefield and Hoffman 1958b) Perhaps the central question concerns the nature of normal repolarization If a prematurely invoked repolarization can propagate, it seems likely that propagation of repolarization exists in the normal heart Since repolarization propagates very slowly (about 0 2 m/sec), one would not look for a single pacemaker from which repolarization would spread over the entire heart What might be expected is that each area which repolarizes before some adjoining area does so would contribute to the repolarization of that adjoining area In that case repolarization would be a propagated

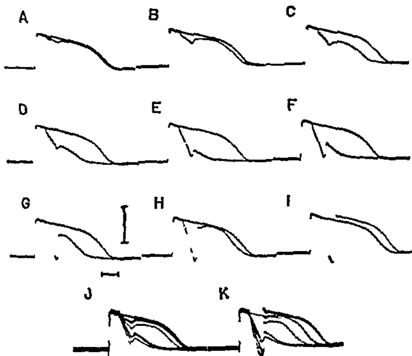


FIG 8-13 The effect of repolarizing pulses on the action potential of a single fiber of papillary muscle in a medium containing 25 per cent of the normal Ca^{++} concentration Gradual increase in strength produces increasing repolarization (A through *E*) followed by increasing break excitation (*F* through *I*) Superimposed traces in the repolarizing ranges are shown in *J* Superimposed pulses in the reexcitation range are shown in *K* Calibrations in *G* time 50 msec voltage 100 mv (*Cranefield and Hoffman* 1958*b*)

process with multiple foci of origin (Cranefield 1957) The significance of anodal regenerativeness in phase 2 is discussed in relation to ionic theories in Chap 9

BIPOLAR STIMULATION

If cardiac excitability is determined by the use of bipolar stimulation (i e by the use of a small anode and a small cathode both placed on the heart) the results are complex As was shown by van Dam and Durrer (van Dam et al 1955 1956) it is quite possible to place a pair of electrodes on the dog heart in such a way that the

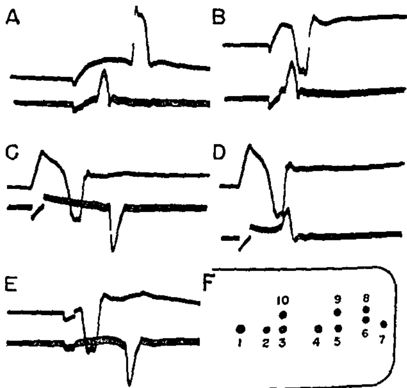


FIG 8-14 Action potentials recorded with the electrode shown in *F* The distance from electrode 1 to electrode 7 is 17 mm In each pair of records the upper trace shows the action potential recorded from electrodes 5 and 6 and the lower trace shows the action potential recorded from electrodes 2 and 3 In *A* *B* *C* and *D* electrode 1 was the cathode and electrode 7 the anode In *A* a stimulus of cathodal threshold strength was applied during diastole activity spread from electrode 1 past electrodes 2 3 5 and 6 in that order In *B* a stimulus sufficiently strong to exceed both cathodal and anodal thresholds was applied during diastole The impulse arising at the cathode spreads to electrodes 2 and 3 the impulse arising at the anode spreads to 6 and 5 in that order The presence of anodal excitation in *B* is shown both by the inverted polarity of the action potential in the upper trace and by the shortened latency of that action potential The records in *C* and *D* are similar to those shown in *A* and *B* except that the stimulus was applied during the dip In *C* therefore threshold is anodal and both action potentials are inverted and the order of latencies is reversed in comparison with *A* In *D* the strength was increased sufficiently to produce simultaneous anodal and cathodal excitation as judged by criteria similar to those used in *B* In *E* electrode 1 was the anode and electrode 7 was the cathode the stimulus was applied during diastole and was threshold for the cathode the action potentials may be compared with those of anodal origin in *A* through *D* (*Cranefield Hoffman and Siebens* 1957)

tissue underlying the anode becomes activated as much as 40 msec earlier or later than the tissue underlying the cathode This means that the excitability curve obtained during bipolar stimulation (cf Figs 8-6 and 8-7) will be dominated by whichever electrode lies on the tissue which repolarized earlier It is possible, by mapping the spread of activation to locate two areas which are activated almost simultaneously and to place the electrodes on those areas However even when this precaution is observed it follows from the discussion of anodal and cathodal excitability given above that the bipolar threshold curve obtained will be composite in nature

The composite nature of the bipolar threshold results from the fact that it is possible to excite the heart with either an anodal or a cathodal stimulus during much of the cardiac cycle (Fig 8 14) It is evident that if both anode and cathode are on the heart, the threshold at any interval will be the lower of the two (anodal or cathodal) thresholds It can be seen (Figs 8-6 and 8 7) that the cathodal threshold is lower than the anodal threshold during phase 4 and during the terminal portion of phase 3 The anodal threshold is lower than the cathodal during the remainder of phase 3 The individual anodal and cathodal curves can be obtained either by recording near the anode and cathode and determining the strength of stimulus necessary to elicit an action potential at one and both or by using a "monopolar cathode or anode A recent paper (Cranefield Hoffman, and Siebens 1957) discusses both methods and also describes the precautions which must be taken in using a "monopolar" electrode

There is reason to believe that various drugs and ions anoxia, and perhaps all agents affect the anodal and cathodal curves selectively It therefore seems probable that determinations of the cardiac excitability cycle and of fibrillation thresholds might be made most meaningful by the determination of both the anodal and cathodal thresholds

THE RESPONSE OF THE HEART TO PROPAGATED ACTION POTENTIALS

Methods

Propagated action potentials can be used to test the recovery of excitability of cardiac cells if advantage is taken of differences in the

action potential duration and of the corresponding differences in the duration of refractoriness found in different fiber types Such differences are prominent at the junction of Purkinje fibers with papillary muscle (Hoffman, Kao, and Suckling, 1957) and at the junction of atrial muscle with fibers of the atrioventricular node (Hoffman, Paes de Carvalho, de Mello and Cranefield 1959) When a fairly abrupt change in fiber type and action-potential duration occurs within a

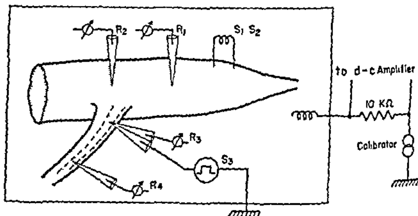


FIG 8-15 Diagrammatic representation of papillary muscle (dense stipple) and attached bundle of Purkinje fibers (light stipple) with single Purkinje fiber indicated by dotted lines S_1 S_2 driving and testing stimuli applied to the papillary muscle through pin electrodes S_3 test pulses applied to single Purkinje fiber through one side of double microelectrode R_1 through R_4 recording microelectrodes located in papillary muscle away from (R_1) and at (R_3) the junction with Purkinje fibers and in a single Purkinje fiber at (R_3) and distant from (R_4) the same junction In some experiments two separate single-lumen electrodes were employed for R_3 and S_3 (*Hoffman Kao and Suckling* 1957)

distance of less than 1 mm it is possible to excite both fiber types and then to excite the more rapidly repolarizing fiber a second time The second action potential of the more rapidly recovering fiber will reach the junctional area at a time when the fiber of the other type is still incompletely repolarized The new response of the partially repolarized fiber is an index of its excitability at the moment of arrival of the second action potential The moment at which the rapidly recovering fiber is excited a second time determines the moment at which its action potential will arrive at the junctional region Thus the excitability of the slowly recovering

fiber can be tested at various times and at the corresponding degrees of repolarization

In the preparation shown in Fig 8-15 the fiber which recovers rapidly is a fiber of the papillary muscle and the slowly recovering fiber is a Purkinje fiber Stimulating electrodes inserted in the distal end of the papillary muscle are used to supply regularly spaced driving stimuli The driven impulses propagate without delay throughout the papillary muscle across the junctional region and

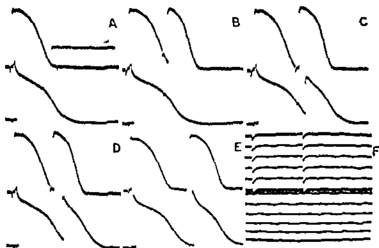


FIG 8-16 Stimulation of Purkinje fibers by action potentials of papillary muscle Transmembrane potentials recorded from single fibers of papillary muscle (top trace) and Purkinje system (bottom trace) Stimulus artifacts show as downward deflection on both traces Both conditioning and testing stimuli applied to papillary muscle Time calibration in *A* in 10 (small pips) and 50 (large pips) msec intervals Voltage calibration in steps of 20 mv shown for both traces in *F* Zero reference level not shown (*Hoffman Kao and Suckling* 1957)

then throughout the length of the attached Purkinje fibers An intracellular microelectrode inserted in a single papillary muscle fiber close to the junctional area indicates the time of arrival of excitation at that point and records the amplitude and configuration of the action potential (Fig 8 16 top trace) Another microelectrode inserted in a single Purkinje fiber some distance from the junction shows (Fig 8-16 bottom trace) the timing and shape of the propagated action potential in Purkinje tissue A third microelectrode is inserted in a single Purkinje fiber near the junctional area to record

the local response of the Purkinje fiber The second, or testing stimulus, which is applied to the papillary muscle at varying intervals after the drive elicits the test action potential The test action potential arrives at the junctional area either during phase 3 or the

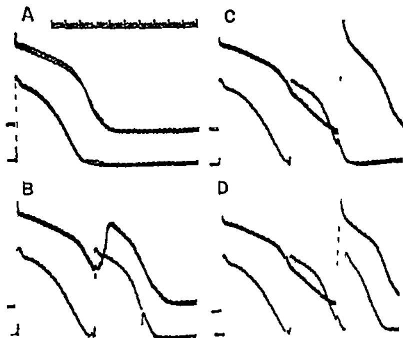


FIG 8-17 Effective refractory period of papillary muscle Records of transmembrane potential of a single Purkinje fiber (top trace) and a single papillary muscle fiber (bottom trace) In *A* and *B* the conditioning and testing stimuli applied to the papillary muscle elicit propagated responses in both tissues In *C* the test action potential of the papillary muscle reaches the *Purkinje* fiber earlier during repolarization and fails to excite Under this condition direct stimulation of the Purkinje fibers gives rise to action potentials which reach the junctional region too early (*C*) or just late enough during repolarization (*D*) to cause a propagated response in the papillary muscle Time calibration in 10- and 50-m ec intervals in *A* Upstrokes of action potentials indicated by dotted lines (*Hoffman Kao and Suckling* 1957)

early part of phase 4 of the driven response of the Purkinje fiber The electrode on the papillary muscle side of the junction indicates the arrival of the test action potential at the junction The electrode at the Purkinje fiber ide of the junction indicates the response of the Purkinje fiber in the near vicinity of the test action potential Since

an apparently large response in the vicinity of the junction may not propagate farther, the electrode in the more distant Purkinje fiber is used to determine whether or not propagation into the Purkinje fiber occurs

A different sequence of stimuli makes it possible to use the same preparation to study the recovery of excitability of fibers in the papillary muscle In this case the test action potential in the papillary muscle fiber is deliberately provoked too soon to excite the Purkinje fiber i e, it is caused to arrive at the junction when the Purkinje fiber is still totally inexcitable The situation is now reversed, the Purkinje fiber proceeds to recover from its first action potential at a time when the papillary muscle fiber is in the middle of its second action potential (Fig 8 17) It is then possible to apply a stimulus directly to the recovered Purkinje fiber at such a time that a test action potential will propagate through the Purkinje fiber and impinge upon partly repolarized papillary muscle

Finally the response of a single fiber to propagated action potentials can be compared to its response to applied cathodal and anodal current pulses by using a double-lumen microelectrode inserted in a fiber located at the junctional area (see below Fig 8 19) (Hoffman, Kao and Suckling 1957 Kao and Hoffman, 1958) One lumen of the double electrode is used to pass pulses of current and the other is employed to record changes in transmembrane potential resulting either from propagated action potentials or applied electrical stimuli

Stimulation of Purkinje Fibers

The most detailed studies of the response of cardiac muscle to propagated action potentials have been carried out on Purkinje fibers because of the relative absence of branching in this system as compared to that found in ventricular muscle and because of the ease with which several electrodes can be placed in a single fiber When a test action potential propagating in the papillary muscle reaches the junction with Purkinje fibers early during phase 3 of the action potential of the latter (Fig 8-18*D*) the junctional Purkinje fiber responds with a small local depolarization which fails to propagate (Fig 8 18*I*) Somewhat later during phase 3 the response of the junctional area is increased in amplitude (Fig 8 18*C*), and either the spatial decrement is less marked or propagation results (Fig 8 18*H*) Still later during phase 3 the response of the junctional

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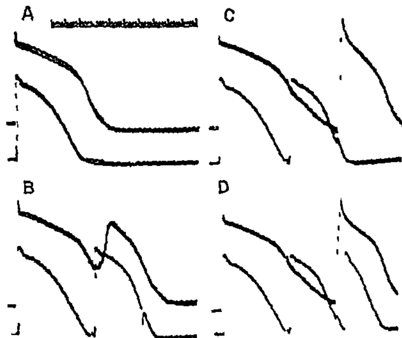


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Fig 8-19 It can be seen that as in other experiments, propagated action potentials of good amplitude and rise velocity result if repolarization is nearly complete (Fig 8-19*B* and *C*) Small, slowly rising but still propagated action potentials arise somewhat earlier (Fig 8-19*D*) Only a decremental response appears when the membrane is stimulated by the test action potential before adequate repolarization has taken place (Fig 8 19*E*) The response of the same fiber to applied cathodal stimuli is shown on the bottom row of the figure Cathodal current pulses give rise to propagated action potentials when the cell has reached a level of membrane potential (Fig 8 19*G*

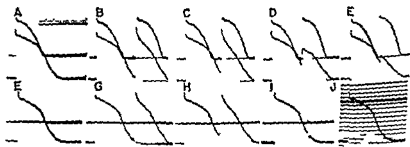


Fig 8-19 Stimulation of a single Purkinje fiber by propagated action potentials of papillary muscle (*A* through *E*) and by cathodal current pulses (*F* through *I*) Note similarity of effective refractory period for both types of stimuli (*C H*) Time calibration in *A* in 10 and 50-msec intervals Voltage calibration in steps of 10 mv and zero reference level (heavy line) shown in *J* See text for discussion (*Hoffman Kao and Suckling* 1957)

and *H*) similar to that which permits a propagated response in response to the propagated action potential The level of membrane repolarization at which applied current pulses are ineffective is about the same as that at which propagated impulses fail (see Fig 8-19*I*) Cathodal stimuli applied at this level of repolarization give rise only to local responses (see Fig 8-23) These results, obtained from stimulation of single Purkinje fibers with both propagated action potentials and applied cathodal stimuli are summarized in Fig 8-20 and are in good agreement with earlier studies by Weidmann (1951) who studied the local response to cathodal current pulses applied during phases 3 and 4

Stimulation of single fibers of papillary muscle through intracellular electrodes gives less conclusive results than does stimulation of the Purkinje system since in the former the syncytial nature of the

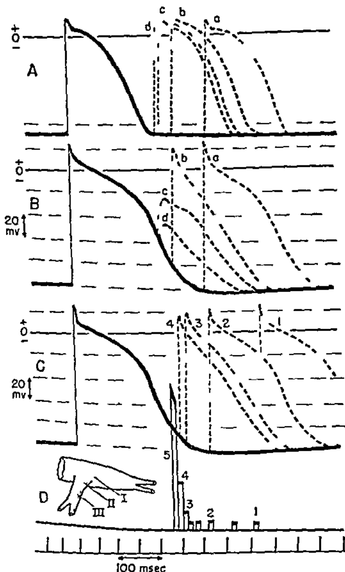


FIG 8-20 Summary of results obtained when a single isolated dog Purkinje fiber is simulated at various times during repolarization by action potentials propagated from attached papillary muscle (4 and B) and by rectangular pulses applied through an intracellular microelectrode (C and D) (A) The transmembrane potentials recorded from papillary muscle at location I in

Fig 8-19 It can be seen that, as in other experiments, propagated action potentials of good amplitude and rise velocity result if repolarization is nearly complete (Fig 8-19B and C) Small, slowly rising but still propagated action potentials arise somewhat earlier (Fig 8-19D) Only a decremental response appears when the membrane is stimulated by the test action potential before adequate repolarization has taken place (Fig 8-19E) The response of the same fiber to applied cathodal stimuli is shown on the bottom row of the figure Cathodal current pulses give rise to propagated action potentials when the cell has reached a level of membrane potential (Fig 8 19G

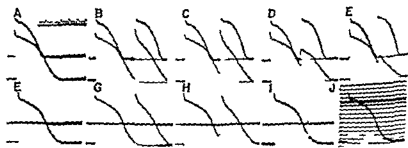


Fig 8-19 Stimulation of a single Purkinje fiber by propagated action potentials of papillary muscle (A through E) and by cathodal current pulses (F through I) Note similarity of effective refractory period for both types of stimuli (C H) Time calibration in A in 10 and 50-msec intervals Voltage calibration in steps of 10 mv and zero reference level (heavy line) shown in J See text for discussion (*Hoffman Kao and Suckling* 1957)

and H) similar to that which permits a propagated response in response to the propagated action potential The level of membrane repolarization at which applied current pulses are ineffective is about the same as that at which propagated impulses fail (see Fig 8 19I) Cathodal stimuli applied at this level of repolarization give rise only to local responses (see Fig 8-23) These results, obtained from stimulation of single Purkinje fibers with both propagated action potentials and applied cathodal stimuli, are summarized in Fig 8-20 and are in good agreement with earlier studies by Weidmann (1951), who studied the local response to cathodal current pulses applied during phases 3 and 4

Stimulation of single fibers of papillary muscle through intracellular electrodes gives less conclusive results than does stimulation of the Purkinje system since in the former the syncytial nature of the

elicits a propagated response at a relatively low strength Also, as the anodal stimulus is placed later and later during repolarization the threshold current requirement increases as shown by the amplitude of the pulses on the top trace. During the latter part of phase 3 anodal stimulation is thus more effective than cathodal and also more effective than anodal stimulation later during phase 3 or during phase 4 A similar period of anodal supernormality has been seen in papillary muscle preparations

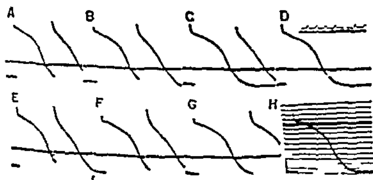


Fig 8-21 Comparison of the effectiveness of cathodal and anodal stimuli applied directly to a single dog Purkinje fiber through an intracellular microelectrode at different times during repolarization Lower trace transmembrane potential upper trace record of stimulus strength (upward deflections depolarizing current downward deflections hyperpolarizing current) Time calibration in D shows intervals of 10 and 50 msec voltage calibration in *H* shows steps of 10 mv See text for discussion (*Hoffman* 1959)

Apart from the phenomenon of propagated repolarization do anodal stimuli play any role in the intact heart? The results of studies on anodal excitation (Cranefield Hoffman and Siebens 1957) have led us to surmise that an infarct might act as an anode with respect to excited tissue The action potential of fibers near the infarct might be shortened and their excitability enhanced Such shortening has in fact been demonstrated (Brooks Gilbert and Mazzella unpublished) The effect of current flow in the vicinity of the infarct has not yet been fully studied It should also be noted that owing to the long duration of the Purkinje-fiber action potential the ventricular fibers probably are anodal with respect to the Purkinje fiber for a time during every cycle (see Fig 8-16)

Graded Response

Applied Stimuli The graded nature of the response of cardiac fibers to stimuli applied during phase 3 of the action potential was first clearly demonstrated by Weidmann (1951) by the u e of single

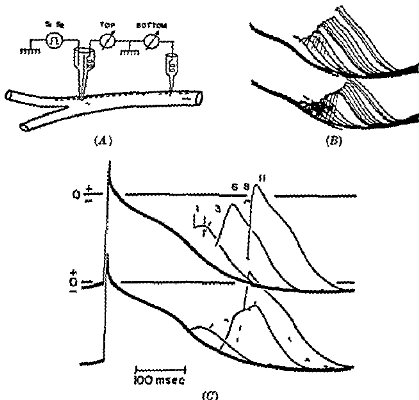


FIG 8-22 Graded respon es in Purkinje fiber elicited by direct stimulation at different intervals during phase 3 (A) Experimental setup, (B) records (C) tracings of selected response in B Separation of the two base lines represents 80 mv see text for discussion (*Kao and Hoffman* 1958)

Purkinje fibers and applied cathodal pulses of relatively long duration Subsequent experiments (Kao and Hoffman, 1958) have been conducted to study the level of membrane potential at which propagation fails in both Purkinje fibers and papillary muscle and to compare the graded response to applied stimuli and to propagated

action potentials In addition, these experiments have provided interesting information on the nature of the increased latency of responses elicited from partially repolarized cardiac muscle fibers

Figure 8 22 shows a series of records obtained from a single isolated Purkinje fiber One side of a double-lumen microelectrode was used for stimulation The response of the membrane was recorded through the other lumen and also through another microelectrode located at some distance from the stimulus site As a cathodal-current pulse of fixed strength was applied progressively later during phase 3, the local response recorded at the stimulus site increased in amplitude rise velocity, and duration The more premature responses show clear decrement at the distal site, the later responses appear at the distant recording electrode as propagated action potentials which vary in rise velocity amplitude and duration One interesting feature of the responses at the distal recording site is the appearance of a second delayed peak which has been interpreted as resulting from excitation at some other more excitable region of the membrane

If the time of application of the stimulus is kept constant and the current strength is varied the results are similar as shown in Fig 8 23 During the latter half of phase 3 the amplitude of the response at the stimulus site is proportional to the strength of the stimulus Weaker stimuli elicit graded depolarizations which show spatial decrement When the amplitude of the local response at the cathode is large enough (Fig 8-23*C* response 8) at the distal recording site there is a slowly rising response which again shows a delayed peak This suggests that the propagated response actually originates at some other area and appears in the record obtained immediately adjacent to the stimulating electrode only as a prolongation in the descending limb of the local response One other aspect of the records shown in Figs 8-22 and 8-23 should be pointed out at this time In both papillary muscle and Purkinje fiber the time of onset of the ultimately propagated response depends in part on the amplitude of the local response Thus, when the stimulus strength is increased above that which is just sufficient to initiate propagation (Fig 8-23, response 8) or applied slightly later than the earliest time during phase 3 permitting propagation (Fig 8 22 response 6) the peak of the response at the distal site appears appreciably earlier in the cycle (Figs 8-23 and 8 22 responses 9 and 11, respectively)

Graded Response to Propagated Action Potentials Use of the papillary muscle–Purkinje-fiber preparation has shown that graded responses, similar to those resulting from cathodal stimuli result when a propagated action potential stimulates an adjacent fiber at the appropriate time during phase 3 Records of this type are seen in

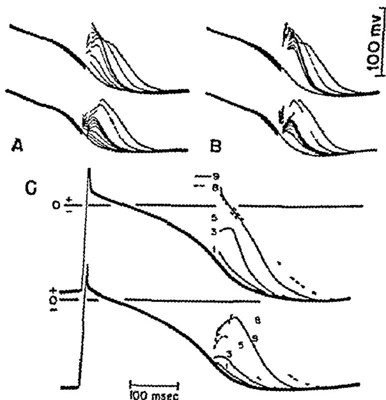


Fig 8-23 Effect of stimulus strength on graded response in a Purkinje fiber Preparation similar to that shown in Fig 8-22 (*A*) and (*B*) records from same preparation (*C*) Selected tracings from *A* Two base lines separated by 80 mv see text for discussion (*Kao and Hoffman* 1958)

Fig 8 18 and are shown more clearly in Fig 8-24 In this experiment the bottom trace shows changes in transmembrane potential of a single Purkinje fiber immediately adjacent to the junction with papillary muscle and the top trace the activity of the same fiber about 2 mm away *from* the junction As the stimulating action potential reaches the junction *later* and later during phase 3 the

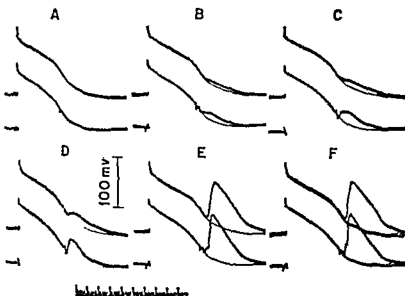


FIG 8-24 Graded responses in a Purkinje fiber Experimental arrangement similar to that in Fig 8-18 except that bottom trace is from the junctional region and top trace from 2 mm away Time calibration 10 and 50 msec Details in text (*Kao and Hoffman* 1958)

response changes from a purely local phenomenon showing spatial decrement (Fig 8-24*C* and *D*) to a local response which culminates in an action potential (Fig 8 24*E* and *F*) The duration of the local response prior to excitation depends upon its amplitude

GENERAL DISCUSSION

Latency

It has been known for many years that in all tissues the latency between stimulation and the appearance at a distance of the propagated action potential changes when stimuli are applied during the relative refractory period Studies on nerve (Forbes Ray and Griffith 1923) demonstrated that this change is due mainly to a change in latency of the appearance of the propagated response and that conduction velocity over most of the fiber length is onl slightly slowed regardless of the time of a the stimulus It has been found (Brooks et al 1955) that the

valid for the heart A clear indication of two mechanisms which contribute to the increased stimulus response interval is shown in several of the preceeding figures First, the time required for the local response to rise to an effective level varies with both the strength of the stimulus and the level of membrane potential during phase 3 Second, the rate of rise and amplitude of the resulting action potential vary and thus influence the rate at which activity spreads in the immediate vicinity of the stimulating electrode An additional factor contributing to changes in latency when stimuli are applied during

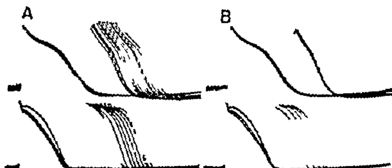


Fig 8-25 Transmembrane action potentials recorded from a single Purkinje fiber (top trace) and a single fiber of attached papillary muscle (bottom trace) Note that as testing stimuli elicit premature responses from the papillary muscle progressively earlier during phase 4 the response of the Purkinje fiber shows a corresponding degree of prematurity only up to a certain level of membrane potential Purkinje fiber responses to earlier activity in the papillary muscle appear at a constant interval during phase 3 and thus show an increasing latency with respect to activity in the papillary muscle See text for discussion (*Hoffman Kao and Suckling* 1957)

phase 3 is seen in some records namely the site at which the propagated regenerative response actually originates As has been demonstrated this point may be either nearer to or more distant from the stimulating electrode

The effect of these factors is magnified when the propagated response travels from a more rapidly repolarizing tissue into one which recovers somewhat more slowly Thus, when activity is recorded on each side of the papillary muscle–Purkinje-fiber junction and testing stimuli are applied to the muscle progressively earlier after the driven response (Fig 8 25), the response of the Purkinje fiber is displaced earlier in the cycle only up to a certain point When the stimuli are still more premature, the respon es of the Purkinje fiber

appear at a fixed time and the latency between the arrival of the action potential at the junction and the appearance of a propagated action potential in the Purkinje fiber increases progressively When the two recording electrodes are both located in the Purkinje fiber one quite close to and the other only 1 mm from the junction the cause of the marked delay is apparent (Fig 8-24) The extent to which these effects may vary because of slight local differences in the action potential duration is appreciable Thus an action potential may rapidly grow to normal amplitude and propagate at normal velocity in one fiber, while in another because of a slightly slower repolarization at a more distant site either delay or complete failure of propagation may ensue Differences in the rate of repolarization may also influence the nature of the response at the junction in the more completely repolarized fiber excitation will start at the junction while in a less fully polarized fiber excitation may occur only at a distance The possibilities for a disorganized response of the intact heart to premature action potentials are thus greatly enhanced by the presence of different fiber types with action potentials of slightly differing durations

The Safety Factor

The safety factor of propagation in cardiac muscle formerly was evaluated by determining the magnitude of threshold change just sufficient to block propagation (see Rothschuh 1952 p 49) This method is liable to error in that any factor producing a major change in threshold may simultaneously alter the regenerative response itself An estimate of the safety factor also may be made from the studies in which the stimulating efficacy of the propagated action potential was compared to that of cathodal pulses of known strength applied at similar intervals during phase 3 In these experiments which are described above the earliest effective cathodal stimulus applied during phase 3 was 4 5 to 6 5 times the diastolic threshold current requirement this observation suggests that the propagated action potential has a safety factor of at least a similar order of magnitude However two factors in these experiments will tend to give too low a value for the safety factor First, the regenerative response of the membrane is actually changed when the fiber is partly depolarized presumably owing to the effect of resting potential on activation of Na^+ carriers (see Chap 9) Second in experiments

in which propagation passes from papillary muscle to Purkinje fibers the progressive increase in fiber diameter would lessen the effectiveness of the stimulus It is likely, therefore, that the propagating action potential in cardiac muscle has a safety factor comparable with that found in nerve (Hodgkin, 1937)

Refractoriness of Cardiac Muscle

Many definitions of refractoriness have been used in describing the recovery of excitability of cardiac muscle A classical division into absolute and relative refractoriness (Brooks et al , 1955) depends upon measurements of response made with recording electrodes at some distance from the stimulating electrodes With this method it is possible to determine an absolute refractory period during which no stimulus can evoke a propagated response a relative refractory period in which strong stimuli can evoke propagated responses, a supernormal period when the stimulus requirement is less than in fully recovered tissue and a period of full recovery in which the threshold is low and constant

Drury (Drury and Love 1926 Drury 1936–1937) defined the effective refractory period as the period which ends at the earliest moment during recovery when a response to a stimulus is conducted throughout the muscle The effective refractory period thus corresponds in duration to the absolute refractory period The term suggests that the muscle is not in fact "absolutely refractory" to the effects of current flow but is merely unable to initiate a propagated response That the heart can show some kind of response during the absolute refractory period was shown by the finding that a stimulus which falls too early to give rise to a propagated action potential may give rise to a prolongation of refractoriness (Drury and Love 1926, Lewis and Drury 1926) More recent work (Cranefield and Hoffman 1958b) suggests that there is no period during the cardiac cycle when a sufficiently long and strong anodal or cathodal stimulus is wholly without influence on the recovery process It can thus be seen that the term *irresponsive period* is not satisfactory unless it is clearly defined in terms of propagated response Drury also defined full recovery time as the moment when delay between test and response becomes constant

If we attempt to summarize the properties of the heart which the above terms are intended to describe we must first note that they

all describe the gross behavior of the whole heart and not the behavior of single cells We find that the following periods can be delineated

1 A period in which no propagated action potential can be evoked by electrical stimulation The tissue is neither 'absolutely refractory' nor 'irresponsive'' during this period since many active changes can be induced by current flow Nor is it strictly possible to subdivide this period into an early part when subthreshold responses are hard to obtain and a late part when they are easy to obtain Although the heart is not absolutely refractory toward electrical stimuli it is 'effectively' refractory in that it will not respond with a propagated action potential It is obvious that the term *refractory* is a very unhappy one but rather than refer to the 'period during which a propagated action potential cannot be evoked by a stimulus even though the stimulus may produce other active responses in the tissue,' it is probably logical to designate this period as the *effective refractory period*

2 The effective refractory period ends at the moment when it becomes possible to evoke a new propagated response by an electrical stimulus Such a response can be evoked only by a strong stimulus and appears only after a considerable latency The period during which excitation can be evoked by a strong stimulus and after a long latency can be said to end at one of two moments the moment when the threshold reaches the value found in phase 4 or the moment when the stimulus-response latency reaches the value characteristic of phase 4 These two moments are not the same The period which begins at the moment when a response can first be evoked and ends at the moment when the threshold reaches the value characteristic of phase 4 is the relative refractory period The moment when the stimulus response latency reaches the value characteristic of phase 4 is called the *full recovery time* It is thus seen that the beginning of the relative refractory period is identical with the end of the effective refractory period but that the end of the relative refractory period need not be the same as the full recovery time

3 In the normal heart the relative refractory period is followed by a brief interval during which the threshold is slightly lower than it is during phase 4 This interval is known as the *supernormal period*

4 For some reason the period following the end of the supranormal period has never received a name which characterizes its

excitability It is phase 4, or *diastole, and in ventricular fibers it is a* period when the cathodal threshold is low and constant and the *stimulus-response interval is low and constant*

It would obviously be desirable to identify the various periods *described above* with the state of a cell as determined by its transmembrane action potential This is unfortunately rather difficult In

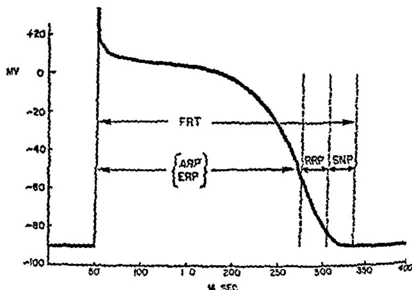


Fig 8-26 Schematic diagram of the usual relationship between *transmembrane* potential and cathodal excitability *FRT* full recovery time *ARP* absolute refractory period *ERP* effective refractory period *RRP* relative refractory period *SNP* supernormal period See text for discussion

the normal fiber a certain relationship usually holds and this relationship is depicted in Fig 8 26 which shows the transmembrane action potential in relation to the different periods The absolute refractory period lasts throughout all of phase 0 phase 1, phase 2, and part of phase 3 The relative refractory period begins when the transmembrane potential reaches a level of about 60 mv The supernormal period appears to be associated with the *terminal part of* phase 3, and the full recovery time ordinarily coincides with the end of the supernormal period and with full repolarization *The greatest* difficulties which arise in establishing this *relationship* concern the end of the relative refractory period and the *full recovery time* This

is so because each depends upon the properties of the cell in a very complex fashion

Threshold depends upon the level of the transmembrane potential and of the threshold potential It also depends upon the cable constants of the fiber Moreover since the relative refractory period as usually determined depends upon the use of external stimulating electrodes the threshold depends upon the fraction of the stimulating current which penetrates the membrane It is therefore impossible to regard the relationship shown in Fig 8 26 as precise Equally, since the full recovery time depends for its definition upon the appearance of normal latency and conduction velocity it cannot be readily related to single-cell properties Conduction velocity is a complex function of all the factors upon which threshold depends and is in addition a function of the swiftness with which regenerative depolarization is able to develop Subject to these reservations the relationship shown in Fig 8 26 can be accepted as a basis for discussion It should be remembered that the threshold to anodal stimuli shows an entirely different relationship to the transmembrane action potential (see Fig 8 21)

The character of the response of a single cell to anodal or cathodal stimuli during the various phases of the action potential was discussed in detail at the beginning of this chapter It need only be said here that the propagated response is always abnormal in rise time amplitude, and propagation velocity throughout all of phase 3 and in particular therefore throughout both the relative refractory period and the supernormal period It is thus the full recovery time and not the end of the relative refractory period which signals the appearance of a fully normal excitability both in the whole heart and in the single cell It has also been pointed out that various agents such as low temperature altered pH drugs and metabolic inhibitors may change the relation hip between the transmembrane potential and the recovery of excitability When this occurs the situation shown in Fig 8 26 no longer obtains

Studies of the excitability of the intact in situ heart as carried out with external stimulation and recording are far easier to conduct than are single fiber studies They may also reveal properties of the whole heart which cannot be revealed by single fiber studies It is apparent on the other hand that such studies will have maximal value if they can be conducted and interpreted in a way which makes

it easy to relate the results to the transmembrane potential (Hoffman, Kao, and Suckling, 1957) The usual descriptions of refractoriness or excitability of the heart fail to take into consideration changes in the response to the applied stimuli This aspect of the recovery of excitability of cardiac muscle appears to be of considerable importance in view of the large safety factor of propagation (Hoffman Kao and Suckling 1957) Indeed, most of the unusual response patterns of partially repolarized cardiac muscle would seem to depend more directly on the altered response (i e the abnormal action potential) than on a change in the threshold to applied electrical stimuli Use of a small suction electrode at the stimulus site will usually give some of the desired information about changes in electrical response without introducing the difficulties inherent in microelectrode recording

9

GENERAL ELECTROPHYSIOLOGY OF THE HEART

An attempt is made in this chapter to review the various properties of cardiac cells from a phenomenological viewpoint and also to examine some theoretical interpretations of the resting potential, pacemaker activity, depolarization, and repolarization. The theoretical investigation is largely limited to the modern ionic theory. It will be seen that the version of that theory presented in Chap. 2 requires many modifications and that its application to cardiac cells remains speculative to a substantial degree.

EXCITATION AND CONDUCTION IN CARDIAC FIBERS

Normal Conduction

There seems to be no reason to suppose that the conduction of the action potential in cardiac cells is fundamentally different from that in most other excitable tissue. All the available evidence suggests that the primary events are electrical, i.e., that propagation depends upon the fact that depolarization at any point in the fiber is regenerative and upon the further fact that the fiber possesses core-conductor properties which ensure that full depolarization at a point in the fiber will induce regenerative and all-or-none depolarization at adjacent areas.

Local Response

Regenerative depolarization which does not develop into a propagated action potential is an important characteristic of cardiac muscle. Not only is it possible to demonstrate a classical local

response to subthreshold depolarization during diastole, but it also appears probable that local responses play a role in the intact heart during phase 3 This is so because the intact heart contains fibers whose action potentials differ in duration from the action potentials of contiguous fibers a fact which permits a second excitation to arrive at a fiber still incompletely repolarized (see Chap 8) A local response may develop under these conditions, and if it does it prolongs refractoriness at the site at which it occurs It is probable that *premature invasion of various parts of the conducting system* by atrial or ventricular impulses does occur and that the resulting local response at the junction with fibers of long refractoriness is important in understanding such phenomena as echoes, the apparent dual atrioventricular conducting systems and the initiation of fibrillation

Threshold Potential

It has been proposed (see Chap 2) that excitation arises when the transmembrane potential of an excitable cell is reduced to a certain critical value the so-called threshold potential This idea is in contrast with the older idea that excitation depends upon reducing the transmembrane potential by a certain amount The evidence for the existence of a threshold potential in Purkinje fibers is quite good, the evidence for other fiber types is less conclusive

All or None Response

In cardiac muscle as in nerve and most types of mammalian skeletal muscle the usual electrical response is all-or none in nature When the membrane potential is lowered sufficiently, regenerative depolarization carries the membrane potential more or less rapidly to a new level which is characteristic of the particular fiber under consideration and which is independent of the strength of the stimulus The *swiftness and magnitude* of the all-or none response are dependent on the fiber type the level of membrane potential prior to stimulation the rapidity with which the membrane potential attains threshold and also on the geometry and membrane potential of fibers just ahead of the active region In addition all the many factors which have been described as modifying the regenerative response also influence the upstroke of the action potential generated by a particular fiber when a constant stimulus is applied at a fixed level of membrane potential

Conduction Velocity

The all-or none response of cardiac muscle is propagated at markedly different velocities in different parts of the heart The extreme values may be as low as 0 02 m/sec in the atrioventricular node and as high as 4 m/sec in the false tendons of the conducting system A good deal of complexity attaches to the interpretation of changes in conduction velocity No complete mathematical formulation relating conduction velocity to the other properties of excitable fibers has ever been presented except that of Hodgkin and Huxley (1952b), and that equation has never been solved explicitly for conduction velocity This is so in part because conduction velocity depends upon so many factors It depends upon the core-conductor properties at rest (which are known with accuracy only for Purkinje fibers) upon the core-conductor properties during activity (which can only be estimated), upon the threshold potential and resting potential and most importantly upon the active response of the membrane which determines the rapidity with which regenerative depolarization develops at an excited area

Changes in the core-conductor properties might account for some changes in conduction velocity as might changes in the threshold potential or resting potential but most changes in conduction velocity probably depend upon changes in the rate of depolarization intrinsic to the excitable membrane The depression of conduction velocity seen in depolarized fibers or in fibers in a medium containing low Na^+ probably results from the fact that the upstroke velocity is reduced It seems quite misleading to explain a slow upstroke velocity as resulting from a slow conduction velocity when in most cases it is actually the slow conduction velocity which is secondary to the slow upstroke velocity This distinction may be clarified by reference to membrane action potentials (Hodgkin and Huxley, 1952b) in which a large area of membrane is synchronously excited In a fiber in which the upstroke of the action potential is slow because the conduction velocity is slow the membrane action potential would be expected to show a rapid upstroke In fibers in which slow conduction velocity is secondary to a slow intrinsic regenerative depolarization the membrane action potential would also show a low upstroke velocity

Although most changes in conduction velocity in a given fiber

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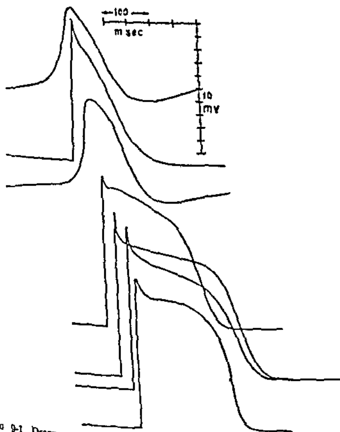


Fig 9-1 Drawings of transmembrane action potentials recorded from the following sites from above down sinoatrial nod atrium atrioventricular node bundle of His Purkinje fiber in a false tendon terminal Purkinje fiber and ventricular muscle fiber Note the sequence of activation at the various sites as well as the differences in the amplitude configuration and duration of the action potentials See text for dis ussion

electrocardiogram thus obtained in relation to the action potentials r corded from single fibers in various parts of the heart and in rela tion to the sequence of activation of these fibers A schematic diagram pre enting information of this sort is seen in Fig 9 1 It is apparent that a knowledge of the voltage time course of activity in each fiber type as well as a knowledge of the conduction velocity and sequen e of depolarization in different parts of the heart can be

employed to aid in the interpretation of the various electrocardiographic waveforms. If this kind of information is available, the appearance of the electrocardiogram may be predicted. It is not usually possible, however, to deduce the shape of the transmembrane potentials and of the sequence of activation from the electrocardiogram. The diagram shown in Fig. 9-1 emphasizes the limited nature and scope of information contained in the usual electrocardiographic tracing. For example, the electrocardiogram rarely contains any indication of the electrical activity of the sinoatrial node, the atrioventricular node, or the specialized conducting system. If electrograms are recorded directly from all parts of the heart, the true sequence of electrical activity can be appreciated, and details such as the proportion of the *PR* interval occupied by conduction in the atrium, atrioventricular node, His bundle, and peripheral Purkinje fibers are revealed (Figs. 7-13, 7-14). However, even records of this sort fail to give adequate information on local differences in the transmembrane potential. Only by correlating studies of the electrical activity of single fibers and groups of fibers with the electrical activity of the entire heart can records of the latter sort be subjected to more precise interpretation.

THE RESTING POTENTIAL

Magnitude

The magnitude of the resting potential of various types of cardiac muscle fibers is listed in Table 3-1. Although many lower values have been reported, the resting potential of most cardiac fibers is between 85 and 95 mv. This range of values is in reasonable agreement with the magnitude of the potential calculated from accepted values for the K^+ concentration gradient and with the predictions of the ionic hypothesis. *In some tissues, such as the sinus venosus* and sinoatrial node, the low resting potential may be associated with a low fiber K^+ concentration and high Na^+ concentration (see Table 5-1).

Effects of Extracellular Ions

The resting potential of cardiac muscle is not changed appreciably if the concentration of Na^+ in the extracellular fluid is increased or if NaCl is replaced by isosmotic amounts of choline chloride or

sucrose Changes in the concentration of Cl^- or of the bivalent cations (Ca^{++} and Mg^{++}) similarly have little effect on the magnitude of the resting potential under ordinary circumstances As in nerve and most vertebrate skeletal muscle the resting potential of cardiac fibers is strongly influenced by K^+ If the concentration of this ion is increased above normal the resting potential falls in linear relation to the logarithm of the K^+ concentration When the K^+ concentration is decreased below normal the resting potential usually fails to increase and often falls to low values Acetylcholine which is thought to increase the permeability of the membrane to K^+, has been shown to bring the resting potential of atrial and sinus tissue closer to the potassium equilibrium potential

Other Factors

If the resting potential is close to the potassium equilibrium potential most agents have little effect on the resting potential or only a delayed effect Thus anoxia substrate depletion and a variety of metabolic inhibitors cause a slow moderate depolarization High concentrations of cardioactive substances such as quinidine and digitalis act similarly Marked cooling for prolonged periods of time causes some depolarization as does extensive stretching

Ionic Basis of the Resting Potential

The potential difference across the resting membrane of an excitable fiber is thought to result from the high concentration of potassium inside the fiber and the tendency of this ion to diffuse outwards to a region of lower concentration If the membrane were selectively permeable to potassium the transmembrane potential should be given by the relationship

$$E = \left(\frac{RT}{F}\right) \ln \frac{[K_i^+]}{[K_o^+]}$$

or, at 37 C,

$$E = 61\ 5 \log \frac{[K_i^+]}{[K_o^+]}$$

At normal extracellular potassium concentrations the resting potential of cardiac muscle fibers closely approximates the value obtained from substitution of measured values for K_i^+ and K_o^+ in the equation above Thus in cat ventricle the fiber K_i^+ is 151 meq/l (Robert

son and Dunihue, 1954), in Tyrode solution containing 2 7 meq/l of K_o^+ the calculated value for the potassium equilibrium potential 104 mv, is reasonably close to that measured by means of intra cellular microelectrodes (85 to 90 mv) In fibers of rabbit atrium the K^+ concentration is 114 meq/l (Walmor Carlos de Mello, unpublished observation), in the Tyrode solution used the K^+ concentration is 2 7 meq/l The theoretical K^+ equilibrium potential therefore is 99 5 mv while the average measured resting potential is 88 mv Part of the discrepancy may result from errors in measurement of the resting potential another source of error is found in the experimentally determined values for fiber K^+ which must depend upon a calculated volume for the extracellular space The difference between the calculated and measured resting potential might also result from potentials contributed by other ions It has been established that the resting membrane is permeable, not only to K^+ but also to Na^+, Cl^-, and Ca^{++}

It seems quite possible that the resting cardiac membrane is indeed almost selectively permeable to K^+ However, until careful measurements of resting potential have been made in Cl^- free media an accurate estimate of relative permeabilities and relative contributions of K^+ and Cl^- to the transmembrane potential cannot be obtained The contribution of Ca^{++} and Mg^{++} to the resting potential can be accepted as negligible, since complete absence of these ions or marked increases in their concentrations are without any appreciable effect at normal values of K_o^+

The changes in resting potential caused by an elevation in the extracellular K^+ concentration also support the concept that this potential is caused largely by the potassium concentration gradient In lower than normal concentrations of K^+ the slope of the line *relating resting potential to log K_o^+ is less steep than that predicted* by the equation given above perhaps because of current carried across the membrane by other ions Another possibility, suggested by experiments on skeletal muscle (R H Adrian, unpublished), is that potassium permeability may be inversely related to the driving force causing K^+ efflux In agreement with either of these possibilities is the finding that the increase in potassium permeability caused by acetylcholine results in an increased resting potential at all levels of K_o^+ and does so particularly at low levels of K_o^+ The effects of Ca^{++} depletion in low K^+ solutions may be brought about

in the same way or through a decrease in inward Na^+ current One other factor influencing membrane potential and presumably also potassium permeability should be mentioned When the resting potential is low, as it is in normal pacemakers or in other fibers which are partially depolarized the transmembrane potential often approaches the potassium equilibrium potential just after phase 3 If phase 3 is indeed associated with an increase in potassium permeability, this observation supports the proposition that the resting potential is determined largely by the potassium concentration gradient and the potassium permeability An alternative explanation for the brief phase of hyperpolarization during and after phase 3 is that it results from inactivation of Na^+ carrier and a transient decrease in inward Na^+ current

It has been mentioned that a variety of factors which might be expected to decrease the active K^+ transport inward across the membrane, either with or without a concomitant change in permeability and passive movement of potassium, do not cause an abrupt fall in the resting potential These findings suggest that metabolic activity contributes to the resting potential primarily if not solely, through the maintenance of a normal concentration gradient of K^+ All available evidence thus suggests that the resting potential of cardiac muscle results from the tendency of K^+ to diffuse from a region of high to one of low concentration across a membrane largely but not exclusively permeable to this ion The major weakness in the experimental evidence supporting this concept stems from a lack of information on the contribution of Cl^- to the resting potential Since Cl^- is in low concentration inside the fiber and in high concentration in the extracellular fluid the potential difference due to this ion would be of the same sign as that due to the K^+ concentration gradient It is to be hoped that future studies will supply information on the movements of K^+ and Cl^- across the resting membrane during phase 3 and phase 4

THE ACTION POTENTIAL

Introduction

In each of the preceding chapters evidence has been presented which bears upon mechanisms possibly responsible for the excitability of cardiac muscle By far the most extensive studies have been

carried out on single Purkinje fibers (Weidmann 1956a), which, because of their large diameter, relatively infrequent branching, and weak contraction, are best suited of all cardiac muscle fibers for experiments of this sort. In summarizing evidence bearing on the nature of the action potential of cardiac muscle, therefore, considerable emphasis will be placed on results obtained from microelectrode studies of Purkinje fibers. Wherever possible, comparable data pertaining to atrial and ventricular muscle will be included for the sake of comparison.

The Applicability of the Hodgkin Hypothesis

The theory of the nerve impulse of the squid giant axon as developed by Hodgkin and Huxley and their associates is complex and well supported. In reviewing the extent to which that theory may be applied to cardiac muscle it is necessary to present a more extended analysis than was given in Chap. 2. Each line of reasoning which is important in the theory as applied to the giant axon will be presented along with the comparable evidence for cardiac tissue.

Impedance Changes. If the action potential is the result of an increased permeability to any or all ion species, it is to be expected that the membrane resistance will fall during the upstroke of the action potential, since the freer movement of ions across the membrane will result in a lower resistance to current flow. The demonstration of this sort of fall in impedance in the squid giant axon (Cole and Curtis 1939) is usually regarded as a classic demonstration of the essential correctness of the view that a change in ionic permeability is essential to propagation of excitation. The finding in itself of course sheds no light on the nature of the ions involved.

Various findings on cardiac cells indicate that membrane resistance falls during phase 0. However, even the most careful study, that of Weidmann (1956b), failed to determine the precise degree to which impedance falls during phase 0. Weidmann found that R_m is low (about 1 per cent of its diastolic value) at the very end of phase 0 and the very beginning of phase 1. It is therefore obvious that R_m falls at least that low during phase 0 but it may fall even lower. Weidmann's studies of R_m were made on Purkinje fibers, and very little useful information is available on the change of R_m during phase 0 in other cardiac fiber types.

External Na^+ *and Action potential Amplitude* One of the earliest observations (Hodgkin and Katz 1948) on which the Hodgkin theory is based was that a quantitative relationship exists between the ratio of Na_o^+ and Na_i^+ and the amplitude of the transmembrane action potential of the giant axon It was found that a lowering of Na_o^+ reduced the amplitude of the action potential without producing a significant change in the resting potential Moreover the reduction of amplitude was found to be in reasonable quantitative agreement with the prediction of the Nernst equation for a potential difference caused by an ionic concentration cell This agreement was found to hold over a rather wide range of external Na^+ concentrations Finally it was noted that a sufficient reduction in Na_o^+ resulted in loss of conduction This fact long known was shown to result not from the total abolition of the regenerative response but from a lowering of its amplitude to a point where the action potential was too small to stimulate adjacent areas

Results in substantial agreement with the Hodgkin hypothesis were obtained on dog Purkinje fibers by Draper and Weidmann (1951) Results on sheep ventricular myocardium are in reasonable agreement with the Hodgkin hypothesis (Délèze 1959) A critical estimate of other results on ventricle will be found in Chap 4 the general conclusion advanced there is that the results do not convincingly support the Hodgkin theory although in most cases they may be interpreted in terms of that theory The most extreme departure from the Hodgkin theory is found in the results of Coraboeuf and Otsuka (1956) on guinea pig ventricle in that tissue the action potential seems able to go on quite nicely in the nearly total absence of external Na^+

It is somewhat disturbing to note (Table 5 1) that the Purkinje fiber the action potential of which behaves in fairly good accord with the Hodgkin theory, departs farther than most cardiac cells from the picture of high Na_o^+ and low Na_i^+ The Purkinje fiber is reported to have a comparatively high Na_i^+ and a comparatively low K_i^+ This fact emphasizes a fundamental difficulty in evaluating results of this sort the paucity of reliable data on ionic distribution in cardiac cells

External Na^+ *and the Steepness of Phase* 0 A decrease in Na_o^+ decreases the driving force acting on Na^+ and also decreases the amount of Na^+ available to carry inward current Both these effects

cause a diminished rate of depolarization during phase 0 This finding is well documented for the squid axon and is fairly well demonstrable in Purkinje fibers (Draper and Weidmann, 1951) sheep ventricle (Délèze, 1959) and rabbit atrium (Hoffman, unpublished) Again Coraboeuf and Otsuka (1956) found that lowering external Na^+ does not diminish the steepness of phase 0 in guinea pig ventricle

Voltage clamp Studies A major line of evidence in support of Hodgkin's explanation of the action potential in the giant axon comes from the so-called voltage-clamp studies In these studies special electrodes and electronic feedback circuits make it possible to displace the resting potential to a new value and hold it there The changes in permeability which occur result in a change in the current flow necessary to hold the membrane potential at a constant value *The current flow is measured and gives an indication of the* permeability changes (Hodgkin, 1957) If the membrane potential is displaced beyond the threshold potential excitation occurs, and the membrane undergoes the sequence of inward and outward current flow which is associated with the initial rise in sodium permeability and with the later increase in potassium permeability It has also been possible in the giant axon, to separate the permeability change into components of sodium and potassium permeability change by clamping the membrane at different voltage levels in *solutions containing different amounts of* Na^+

Further important information about the Na^+ permeability changes is obtained by the two-step clamp experiments In these experiments the membrane is clamped at some level, and then the voltage level is suddenly shifted If the initial level was one which produced an action potential the effect of a new clamp at a level nearer the resting potential is to turn off the action potential to a greater or lesser degree Experiments of this sort give information on the effect of repolarization on Na^+ permeability and also reveal the *intrinsic fall in* Na^+ *permeability which results* automatically from depolarization (the inactivation of Na^+ permeability as a result of depolarization)

If the initial level at which the membrane is clamped is not one which results in excitation and the second *level is one which does* information is obtained on the effect of preliminary *depolarization* or hyperpolarization on the subsequent action potential If the membrane is partially depolarized for a time before the action

potential is evoked it is found that inactivation of the Na^+ carrier system has occurred This inactivation manifests itself in a lowered degree of Na^+ permeability increase during the action potential Such inactivation corresponds to the lowered amplitude and rise rate of an action potential propagating through a partially depolarized fiber Hyperpolarization on the other hand, increases the degree to which Na^+ permeability increases during an action potential This corresponds to the old observation that an action potential which propagates through a hyperpolarized region does so with increased velocity (faster upstroke) and increased amplitude

Weidmann (1955*a* *b*) has carried out experiments of a similar sort on single Purkinje fibers by applying polarizing current through one intracellular microelectrode and recording the transmembrane action potential through another microelectrode inserted into the same fiber at a short distance from the polarizing electrode In these experiments the transmembrane potential close to the polarizing electrode could be maintained at any desired value by means of a feedback circuit However since only a small portion of the membrane was clamped at the desired potential measurements of the membrane current were not obtained Instead changes in availability of Na^+ carrier were inferred from changes in the rising velocity of the action potential upstroke and from changes in the magnitude of the overshoot

When the membrane of the Purkinje fiber was clamped for 50 to 100 msec at different potentials and then stimulated an S shaped relationship between the clamp potential and rate of rise of the action potential was obtained The rate of rise was maximal at a transmembrane potential of 90 mv or higher was half maximal at 71 mv and fell to zero between 40 and 50 mv Also the overshoot was maximal when the membrane was clamped at 90 mv and fell to zero at clamp potentials of 60 mv When the same studies were made in a solution containing only 25 per cent of normal Na^+ a similar S shaped curve was obtained however maximal rate of rise was only 37 to 57 per cent of that seen in normal solution The relationship between the membrane potential and the rising velocity of the action potential was the same when the membrane was clamped at the desired level during diastole as when the clamp voltage was applied during the action potential Similarly, when the membrane was depolarized by excess KCl an increase in membrane

potential due to the voltage clamp restored rising velocity of the action potential to values comparable to those obtained at similar clamp voltages in normal solution Double pulse experiments similar to those described for the squid giant axon demonstrated that changes in the rising velocity of the action potential followed a change in membrane potential more rapidly when the change in membrane potential was large The time constant for changes in the rate of rise was found to be less than 20 msec

These experiments can be interpreted to mean that changes in the maximum rate of rise of the action potential are indicative of changes in the sodium permeability of the membrane and of changes in the inward sodium current density In this sense they are also a measurement of the availability of Na^+ carrier and can be compared to the results obtained by Hodgkin from squid giant axon In general there is good agreement between the measurements made on the two types of tissue Studies of other types of cardiac muscle also suggest that there is a similar relationship between the level of membrane potential prior to stimulation and the rate of rise of the action potential and amplitude of the overshoot When papillary muscle fibers are repolarized by pulses of anodal current applied at any time after phase 0 (see Chap 8), the maximum rising velocity and amplitude of the break response depends on the level of membrane potential prior to the break in much the same manner as shown in the voltage-clamp experiments When the resting potential of cardiac fibers is lowered by a variety of factors such as excess KCl (Fig 3 9) or local pacemaker activity (Fig 7-4) there is a decrease in the rising velocity and in the amplitude of the action potential

Since it has not been possible to effect a true ' clamp ' of a cardiac cell, it has not been possible to determine the true change in permeability during activity, nor has it been possible to separate the permeability change into changes attributable to specific ions Such a separation in the squid giant axon provided the basis for a mathematical synthesis of the action potential solely in terms of voltage and time dependent permeability changes (Hodgkin and Huxley 1952b) A reconstruction of this kind obviously has not been possible for cardiac cells

Ion flux Measurements If the action potential results from an increase in Na^+ permeability and Na^+ influx and a subsequent increase in K^+ permeability and K^+ efflux, each action potential

should result in a net gain of Na^+ and loss of K^+ by the fiber If the passive electrical properties of the excitable membrane are known, it is possible to estimate the magnitude of the ionic fluxes associated with an action potential Studies of giant nerve fibers have demonstrated that during activity there is a net gain of Na^+ and loss of K^+ and also that the magnitudes of the net fluxes determined by the use of radioactive tracers are in good agreement with the values obtained from calculations based on the ionic hypothesis (Keynes 1951a, b) Other studies of giant nerve fibers have shown that after a period of activity, there is an active extrusion of Na^+ and uptake of K^+, so that the normal concentration gradients of these ions are maintained (Hodgkin and Keynes 1954, 1955) This active transport of ions can largely be inhibited by certain agents such as dinitrophenol azide or cyanide, however, even when Na^+ extrusion and K^+ uptake are markedly depressed by such metabolic poisons the action potential is unchanged in amplitude and form and the uptake of Na^+ during activity is unaltered The conclusion obtained from experiments of this sort is that metabolic activity is important in maintaining the normal concentration gradients of Na^+ and K^+ and in promoting recovery from activity but that metabolic reactions of this sort are not directly involved in the production of the action potential

A number of studies of several types have shown that under appropriate conditions activity of cardiac muscle is associated with a net loss of K^+ and gain of Na^+ In general however if isolated preparations of heart muscle are in good condition and are in an appropriate environment there is no demonstrable net change in the ionic content of the fibers even after prolonged periods of activity at reasonably rapid rates Moreover it has not been possible to demonstrate a net gain of Na^+ or net loss of K^+ during a single cardiac action potential The most suggestive experiments of this sort are those of Wilde (1957), using a turtle heart previously loaded with radioactive K^+ he demonstrated a periodic efflux of K^+ which appeared to be synchronous with repolarization If preparations of cardiac muscle are treated with any of a large variety of metabolic inhibitors (see Chap 4) there is a decrease in the resting potential and in the amplitude of the action potential and frequently a decrease in the duration of the action potential as well However, all of the early changes in the action potential may well result from the

effect of the altered resting potential on inward Na^+ current. When metabolic inhibition has existed for longer times, there is little doubt that the ionic concentration gradients are altered and that the fiber gains Na^+ and loses K^+. This observation supports the obvious conclusion that the maintenance of the normal concentration gradients depends ultimately on some aspect of cellular metabolism; however, in the heart, as in giant nerve fibers, there is no evidence in support of the idea that the action potential itself is directly dependent on any of the metabolic pathways which have been blocked by inhibitors.

Summary

It will be seen that the evidence in support of the applicability of the Hodgkin hypothesis to cardiac cells is less convincing than is that which supports the applicability of the theory to the giant axon. It is interesting to note that the few quantitative studies available all have one thing in common. Reduction of external Na^+ alters the action potential, but it does so to a lesser degree than the theory predicts. This fact suggests the possibility that some other ion participates in the depolarization. It seems extremely likely that an increase in P_{Na} plays a significant role in the process of depolarization in most cardiac fibers; it also seems likely that some additional unknown factor is important.

REPOLARIZATION

Introduction

Most of the peculiarities which distinguish action potentials of cardiac cells from those of other excitable tissues are associated with the delayed and slow repolarization which is seen to a greater or lesser extent in records from all cardiac fibers. The duration of the action potential of cardiac cells varies within the same heart as well as from one species to another, but it is usually long in comparison with the action potential of nerve or skeletal muscle of the species in question. This sustained depolarization may be important in maintaining the prolonged contraction characteristic of heart muscle; moreover, most of the curious aspects of cardiac excitability are seen during the period of repolarization. Because this aspect of the electrical activity of cardiac fibers is not only unusual but also

poorly understood the effect of a variety of agents on repolarization will be summarized below In the last section of this chapter an attempt is made to relate the various known facts to an ionic theory The summary of the effects of various agents is oriented toward the theoretical discussion and is not therefore exhaustive No detailed comparison with the giant axon will be made since it is clear that the mechanism of repolarization in cardiac cells differs significantly from that of the giant axon

The Phases of Repolarization

It seems advisable at this point to examine critically the division of repolarization into three phases This division is used because it is convenient for descriptive purposes Phase 2 corresponds to the plateau and phase 3 to the phase of rapid repolarization When one speaks of the slope or steepness of phase 1 2 or 3 one refers to the slope of a line more or less tangent to the action potential during the phase in question This tangent does not represent in detail the actual voltage change Among other things important transitions are ignored completely These transitions, from phase 1 to 2 from phase 2 to 3 and from phase 3 to 4 are of considerable interest For example cooling changes the transitions as much as or more than it changes the slopes of phases 2 and 3 In general any agent which affects the time course of repolarization alters the whole shape of action potential To describe the effect of temperature or some other variable solely in terms of the slopes of phases 1, 2 and 3 is to suggest that nothing has changed but the time axes of these phases Finally it should be emphasized that certain properties of the membrane such as impedance change continuously during any particular phase The distinction between the three phases may or may not be a fundamental one in any case the nomenclature will be used in this chapter as it is in the rest of the book

The Presence of Three Phases in Different Tissues In spite of the above reservations it is important and interesting to note that nearly all excitable tissues display at least two phases of repolarization which correspond to phases 2 and 3 A slow and fast phase of repolarization may be seen e g in the isolated node of Ranvier and in skeletal muscle As far as cardiac tissue is concerned the presence or absence of the various phases has been discussed in the chapters on the fiber types Broadly speaking all three phases are present in

the Purkinje fibers and ventricular fibers of large mammals, phase 1 is often absent in atria and at low temperature in frog and turtle ventricle, phase 2, though present is steep and brief in the atrium of most species and in the ventricle of some small mammals

Temperature

A critical examination of the effect of cooling on the cardiac action potential immediately reveals the inadequacy of a description of the changes produced by low temperature phrased only in terms of the temperature coefficient of the slope of the three phases of recovery It is certainly true that the great majority of the prolongation results from a decrease in the slope and therefore a lengthening, of phase 2 On the other hand extreme cooling prolongs phase 3 rather drastically Moreover transitions between the phases become markedly slowed, and something almost like a new phase may appear between phases 1 and 2 Much information doubtless remains to be obtained by new and detailed studies of temperature effects The work of Heintzen suggests that neither the division into three phases nor the use of a simple Q_{10} is adequate to describe effects of temperature on the action potential In the meantime the pertinent observations can be summarized by stating that cooling greatly prolongs the duration of the action potential in all fiber types from all species and that phase 2 is more sensitive to temperature change than are phases 1 and 3 (see Chap 4)

Rate

Increasing the frequency of activity in cardiac tissues decreases the duration of the action potential A decrease in rate resembles a decrease in temperature in that the observed prolongation may largely be attributed to an increased duration of phase 2 The rate effect is a strong one In some fibers the action potential duration may be reduced by a sufficient increase in rate to less than one-third of the duration seen when rate is "infinitely slow" All studies involving rate changes are subject to certain reservations which are discussed in detail in Chap 4

It seems probable that in any particular cardiac cell there is under any given set of conditions a rate which is so slow that further slowing will not produce any increase in duration The duration at such a rate is therefore presumably determined solely by the general

environmental factors such as temperature and ionic milieu acting in conjunction with whatever mechanisms are reponsible for repolarization When rate is changed the new duration does not immediately reach a steady state The shortening seen with acceleration requires a number of beats to stabilize, while the prolongation seen with slowing may require twenty or thirty beats or longer to become fully established An extreme increase in rate may abolish phase 1 entirely and even result in each new action potential arising before full repolarization of the preceding action potential Acceleration of such degree markedly changes the entire configuration of all phases of the action potential

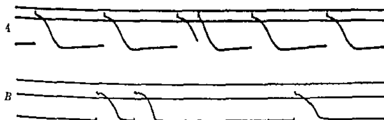


Fig 9 2 Records of transmembrane potentials recorded from a single Purkinje fiber showing some depolarization during phase 4 (above) and from a single fiber of papillary muscle (below) In both sets of records the top trace shows time marks at intervals of 50 and 500 msec Note the configuration of the single extrasystole in each record See text for discussion

Premature Beats If the tissue under study is stimulated at a regular slow rate it is of interest to note the configuration of the action potentials of single interpolated extrasystoles A number of such early extrasystoles are shown in Figs 8 18 and 8 22 When the extra action potential arises so early that phase 0 is slow and the amplitude is reduced the duration is markedly reduced It will be seen in Fig 9 2*A* that a moderately early extrasystole which produces a reduction in duration is followed by an afterhyperpolarization (fourth beat) Finally a beat interpolated midway between two other beats shows a remarkable change in configuration (Fig 9 2*B*) the action potential is more square, i e phase 2 is rather flat and the transition between phases 2 and 3 is rather abrupt

Ions

Sodium It has been noted that a marked reduction of external Na^+ concentration results in shortening of total action potential

duration whereas an increase of external Na^+ results in lengthening. This generalization unfortunately is not a comprehensive one. Two variables appear at present to be most important in determining the exceptions: Species variation and variation in the substance used to replace NaCl. The need for systematic experiments on a single species and tissue type designed to show clearly the effect of a variety of NaCl substitutes is indicated by results summarized in Table 9-1. The effect of sodium depletion on action potential duration depends in part on the residual concentration of Na^+. In studies of rabbit atrium (Fig. 3-8) moderate reduction of Na^+ may cause some prolongation; further lowering of Na^+ concentration then leads to the shortening usually observed.

TABLE 9-1. THE EFFECT OF SODIUM DEPLETION ON ACTION POTENTIAL DURATION

Species and tissue	NaCl replaced by	Percentage replaced	Effect on duration	Reference
Dog Purkinje fiber	Sucrose	87	Reduction	Draper and Weidmann 1951
Snapping turtle Ventricle	Sucrose	66	Reduction	Cranefield, Eyster and Gilson 1951a
Frog Ventricle	Sucrose		Increase	Brady and Woodbury (195[illegible])
Frog Ventricle	Choline chloride		Decrease followed by increase	Brady and Woodbury (1957)
Guinea pig Ventricle	Choline chloride	25	Increase	Coraboeuf and Otsuka (1956)
Guinea pig Ventricle	Choline chloride	50	Prolongation followed by reduction	Coraboeuf and Otsuka (1956)
Guinea pig Ventricle	Choline chloride sucrose		Prolongation	Délèze (1959)
Sheep Ventricle	Sucrose or choline chloride	75	Reduction	Délèze (1959)
Rabbit Atrium	Sucrose or choline chloride	75	Reduction	Hoffman and Cranefield, unpublished

The cholinergic action of choline chloride is only 0 001 per cent as great as that of acetylcholine Nevertheless there is no reason to regard choline chloride as an inert substitute for NaCl this fact is particularly important in studies of cardiac tissues On the other hand substitution of NaCl by sucrose results in replacement of both Na^+ and Cl^- It is clear that much work needs to be done in this area

Potassium Increase in external K^+ shortens the duration of the action potential This effect results in part from a reduction in the resting potential On the other hand application of a high concentration of KCl to fibers of the turtle ventricle during phase 2 causes a marked curtailment of this phase without prior reduction of the resting potential Weidmann has found anodal polarization to be ineffective in counteracting this effect (Shanes 1958) Weidmann has also shown that anodal repolarization of fibers depolarized by a high extracellular concentration of K^+ restores the amplitude of phase 0 relatively more than it restores the duration of phases 2 and 3 It thus appears that the effect of K^+ on duration results only in part from the concomitant reduction in the resting potential Attention should be called to the results of Wilde which suggest that there is a substantial increase in the efflux of K^+ from cardiac cells during either phase 2 or phase 3 (see Chap 4)

Calcium Reduction of the extracellular Ca^{++} concentration increases the duration of the action potential of ventricular fibers chiefly by increasing the duration of phase 2 Phase 2 occurs at a level somewhat closer to the resting potential than it does in the normal fiber Similar reduction in Ca^{++} has little effect on the action potentials of atrium or Purkinje fibers Elevation of Ca^{++} results in reduction or disappearance of phase 2 in both papillary muscle and atrium In atrium although phase 2 is steep phase 3 is slow in the presence of excess Ca^{++} Elevation of Ca^{++} has little effect on the action potentials of Purkinje fibers

Acetylcholine

Ventricle and Purkinje Fiber These tissues are extremely insensitive to the action of acetylcholine and vagal stimulation Nevertheless, very high concentrations of acetylcholine have been reported to shorten the action potential of frog ventricle and of Purkinje fiber These actions occur at concentrations so different from those which reproduce the effects of vagal stimulation on atrium that they may

perhaps be ignored completely In any event one should not assume that the mechanism by which these changes are produced is similar to that postulated to explain the effect of acetylcholine on atrial fibers

Sinoatrial Node and Atrioventricular Node The principal effects of acetylcholine on these fibers are on phases 0 and 4 In general, however, nodal fibers show some slight shortening of action potential duration at concentrations of acetylcholine high enough to reduce greatly sinoatrial nodal pacemaker activity or cause atrioventricular nodal block

Atrium Acetylcholine causes a remarkable shortening of the action potential of atrial fibers in all species which have been studied In the presence of an adequate concentration a virtual abolition of phase 2 occurs and in fact the voltage time course of repolarization is fundamentally changed (Fig 3 5) The succession of fast, slow, and fast phases represented by phases 1, 2 and 3 seems to vanish and to be replaced by a simple continuous change in membrane potential in which repolarization resembles an exponential decay proceeding most rapidly initially and less and less rapidly as time goes on The last 20 per cent of repolarization is actually quite slow, but the earlier part is so fast that total duration is vastly reduced Transitional shapes can be seen at intermediate concentrations of acetylcholine Evidence from various sources strongly suggests that acetylcholine increases the permeability of the atrial cell to K^+ Such an increase would be in accord with the observed effect on repolarization

Epinephrine

Acetylcholine affects the slope of phase 4 in pacemakers and the velocity of repolarization in atrium Epinephrine affects the slope of phase 4 in pacemakers in manner opposite to acetylcholine, but its effect on repolarization in atrium or other types of cardiac fibers is by no means as marked nor is it as obviously antagonistic to the acetylcholine effect If epinephrine leads to an increased rate, rate-induced shortening occurs If acceleration does not occur, the effect of epinephrine on repolarization depends on the species and tissue Table 9 2 shows results from experiments in which rate change supposedly did not occur

TABLE 9-2 THE EFFECT OF EPINEPHRINE ON THE DURATION OF THE ACTION POTENTIAL

Species	Tissue	Effect	Reference
Turtle	Atrium	None	Churney (1952)
Rat	Atrium	Prolongation	Webb and Hollander (1956a)
Dog	Atrium	Shortening	Brooks et al (1955)
Frog	Ventricle	Prolongation	Lucken and Schütz (1938)
Dog	Purkinje fiber	Slight prolongation	Cranefield and Hoffman unpublished

Resting Potential

It seems to be quite generally true that, when the resting potential is reduced, not only are the steepness of phase 0 and the amplitude of the action potential reduced but total duration also is decreased generally at the expense of phase 2 So many of the agents which reduce the resting potential probably also alter the K_i^+/K_o^+ ratio that it is difficult to separate a possible K^+ effect from the effect of the resting potential per se

Cathodal Stimuli

Pulses of cathodal current which are reasonably short compared with the duration of the action potential are without marked active effect during phase 1 or 2 although the change in transmembrane potential is not wholly passive During the latter half of phase 3 cathodal stimuli either give rise to local nonpropagated depolarization or to action potentials of low rise velocity reduced amplitude and short duration

Weak Anodal Stimuli

Anodal current pulses which are short with respect to the duration of the action potential displace the transmembrane potential somewhat more during phase 2 than do cathodal stimuli Active responses to anodal stimuli during phase 3 include the appearance of propagated action potentials at stimulus strengths lower than are needed for cathodal excitation Propagated action potentials can be elicited by anodal stimuli early in phase 3, at which time no propagated responses can be elicited even by very strong cathodal stimuli

Strong Anodal Stimuli

Strong anodal current pulses applied during phase 2 are capable of inducing all-or none repolarization In ventricle this all-or none repolarization often terminates in reexcitation after the end of the anodal stimulus If, however the fiber is cooled or subjected to low Ca^{++}, break reexcitation is less readily obtained, and instead a sustained repolarization results which may give rise to propagated repolarization Sustained and propagated repolarization without break reexcitation is more readily obtained in normal Purkinje fibers than in ventricular muscle Strong anodal stimuli applied during phase 3 may fail to excite at the same interval when weaker or shorter anodal stimuli give rise to propagated action potentials—*this is the no response phenomenon*

Separation of Repolarization and the Recovery of Excitability

The fact that full repolarization does not necessarily imply full recovery of excitability is of great interest Such a situation has been found in studies of the action potential of frog heart under certain conditions and has been shown to play a role in the action of quinidine It is also seen in the artificial situation described above in which cold or lowering of Ca^{++} reduces the likelihood of anodal break reexcitation following strong anodal stimuli applied during phase 2 It is also possible that recovery of excitability lags behind repolarization in normal fibers of the sinoatrial node

Anodal Polarization

Continuous anodal polarization and anodal polarization initiated at the end of phase 0 and continued on into the next phase 4 both produce marked reduction in the duration of the action potential (Fig 9 3) This reduction in duration is very great when compared with the hyperpolarization seen in the succeeding phase 4 and may be observed in the almost complete absence of such *hyperpolarization*.

Cathodal Polarization

It is possible to "clamp" the transmembrane potential at various levels of depolarization by applying suitable current (Weidmann 1956a) When the transmembrane potential is thus changed (Fig 9-4), there is no evidence of an impedance change during the

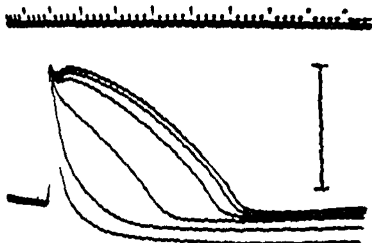


Fig 9-3 The effect of anodal polarization on the action potential of papillary muscle The anodal pulse is turned on immediately after the end of phase 0 Superimposed sweeps show effects of increasing current strength Note the marked acceleration of repolarization which results from current strengths which cause only slight hyperpolarization during diastole (*Cranefield and Hoffman* 1958b)

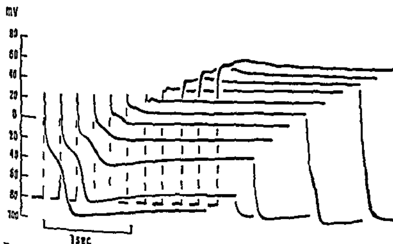


Fig 9-4 Tracings of the transmembrane potential of a single isolated Purkinje fiber showing the effects of depolarizations of long duration brought about by clamping the membrane potential at various levels for an interval of 2 sec. See text for discussion (*Weidmann* 1956b)

"clamp," and there is therefore no delayed rectification of the sort seen in the squid axon as the result of an increase in P_K. This means that the mere passage of time, combined with a given level of depolarization, does not result in an increase in K^+ permeability. It is of interest that, when the "clamp" is released, the transmembrane potential rapidly falls, probably with the time constant of the membrane, to the approximate potential at which phase 3 ordinarily begins, and repolarization then proceeds along a voltage time course closely resembling that of phase 3.

Impedance

The electrical impedance of the cell is supposed to bear a relation to the permeability of the membrane to ions. When the impedance is high, the ionic permeability is generally assumed to be low. The observation, therefore, that the impedance of the cardiac fiber is high during phases 2 and 3 is of great importance to ionic theories of repolarization. All theories advanced to date take Weidmann's observation on the impedance of the Purkinje fiber as a starting point. Further studies of impedance with various methods and on as many other cardiac tissues as possible are urgently needed but such studies are unfortunately very difficult and indeed potentially somewhat treacherous.

Weidmann (1951) determined the impedance change of a single Purkinje fiber during phases 1, 2, and 3 with a technique using intracellular electrodes. One electrode was used to apply pulses of anodal current to the membrane, and the other was used to record the resultant changes in membrane potential. An experiment of this type indicates changes in impedance, since the higher the membrane resistance, the greater is the potential change across it in response to a current pulse of constant amperage. Weidmann found (Fig. 7.17) that during phase 1, R_m is lower than in phase 4 and during phases 1 and 2 it rises fairly rapidly and steadily until it reaches a value more than three times that observed during phase 4. He also observed a marked and rather rapid drop in R_m during phase 3. These observations are in general agreement with those of earlier workers who, using less satisfactory methods, found an impedance rise during phase 2 (see Chap. 4).

Somewhat similar results have been obtained in studies of guinea pig ventricle (Coraboeuf, Zacouto, Gargouil, and Laplaud 1958). It

was found that impedance ri es during phase 2 and drops during phase 3, but R_m is never as large during phase 2 as during phase 1

Changes in the membrane impedance of single fibers of rabbit atrium have recently been studied by recording the displacement of

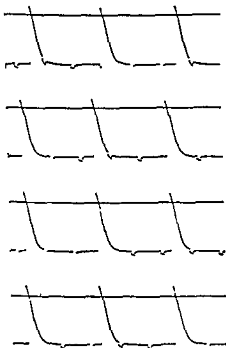


FIG 9-5 Records of the transmembrane potential of a single fiber of rabbit atrium recorded differentially between two microelectrodes one inside and the other just outside the membrane showing the displacement of membrane potential produced by constant-current anodal pulses applied at various times during phases 0 to 4 The short segment of record at the bottom shows the potential change produced by current pulses of the same strength when both recording ele rodes are located just outside the membrane See text for discussion

transmembrane potential caused by pulses of anodal current applied at different times during phases 0 to 4 (Cranefield and Hoffman unpublished ob ervations) The results obtained (Fig 9 5) suggest that there is a marked drop in R_m during phase 0 and that membrane resistance increases progressively during phases 2 and 3 and finally attains a steady value early in phase 4

Recent studies of dog atrium (*Trautwein and Dudel, 1958a*, Matsuda, personal communication) indicate that the action of acetylcholine is accompanied by a definite reduction of impedance during repolarization This observation is in accordance both with the K^+ permeability increase which occurs when acetylcholine is added to atrial tissue and with the fall in impedance during phase 4 which results from the action of acetylcholine The most important question regarding the action of acetylcholine on atrial fibers is whether the action on impedance is specific or nonspecific as far as the different phases are concerned If one supposes that repolarization of atrial cells is accompanied by a rise in P_K and a fall in R_m during phase 3, it does not follow that acetylcholine enhances these changes On the contrary, the effect of acetylcholine may be to increase P_K to a high level which remains relatively constant throughout the entire cycle If that is the case the action potential in atrial fibers exposed to acetylcholine might depend solely upon cyclical changes in P_N

Regenerative Repolarization

The highly nonlinear response of the transmembrane potential to anodal stimuli applied during phase 2 suggests that repolarization is a regenerative process A variety of observations argue for regarding phase 2 as a pacemaker and phase 3 as a phase of *active regenerative* repolarization These observations are summarized in Chap 8 and in Cranefield and Hoffman (1958b) From this point of view the level *of transmembrane potential at* which phase 2 ends and phase 3 begins may be regarded as a threshold for repolarization The existence of such a threshold would imply that, if a sufficiently large area of membrane were to be held for a sufficiently long time at the threshold for repolarization repolarization would then continue to completion much as regenerative all-or none depolarization follows a threshold depolarization

Potassium Flux

There is general agreement that K^+ leaves the cardiac cell during activity and that acetylcholine enhances K^+ *permeability* in atrium The principle point of disagreement is to what extent K^+ exit corresponds in time to the phase of rapid repolarization On this point disagreement is complete, some authors *claiming* that all of the

excess K^+ efflux associated with activity depends upon an increase in K^+ permeability during phase 3, while others claim no increase whatever is needed to explain the K^+ loss associated with activity

Inhibitors

Most if not all inhibitors act as does anoxia to produce a shortening or loss of phase 2, so that fibers whose action potentials ordinarily show a well marked plateau begin instead to show an action potential rather like that of atrial fibers The most obvious interpretation of these observations is that metabolic inhibitors and hypoxia damage the membrane with the expected consequence that K^+ permeability increases

A POSSIBLE IONIC MECHANISM FOR REPOLARIZATION

Introduction

If the transmembrane potential E_m in cardiac fibers is assumed to represent primarily the sum of an emf resulting from a Na^+ gradient E_N and an emf resulting from a K^+ gradient E_K and if the relative contribution of each emf is assumed to depend upon the permeabilities to those ions P_K and P_N and if the observation that impedance is high during phase 2 is accepted as correct then the following statements are probably correct

1 During phase 2 the transmembrane potential is near zero This means that the contributions to the transmembrane potential of E_N and E_K approximately balance one another and therefore that P_N and P_K are of the same order of magnitude

2 The fact that the membrane resistance is high indicates that P_K and P_N are both rather low

3 The fact that the transmembrane potential shifts slowly towards E_K during phase 2 indicates that P_N is falling or that P_K is rising or that a Na^+ extrusion mechanism with the characteristic of a charge pump is operating

Various theories have been advanced to explain these observations (Weidmann 1956b Cranefield and Hoffman 1958a) None has proved to be completely satisfactory Our opinion in 1957 (Cranefield and Hoffman 1958a) was

A simple hypothesis for the mechanism of repolarization may be formulated as follows during phase 2 the potential gradient permits outward K^+ movement but K^+ permeability is low, perhaps lower than in diastole As the concentration of K^+ rises outside the fiber it enhances K^+ permeability When a certain level of outside K^+ is reached (which would ordinarily correspond to a certain ratio of K_i^+ to K_o^+ and therefore to a certain membrane potential) K^+ permeability begins to rise more rapidly initiating phase 3 If external K^+ is artificially increased the expected result would be an increase in the slope of phase 2 and a decrease in the slope of phase 3

The hypothesis that the change in potential which occurs during phase 2 of the recovery process is analogous to the depolarization observed during diastole in a pacemaker cell and that the change in phase 3 is an active regenerative depolarization which begins when a certain level of membrane potential is reached is a very attractive one On the basis of such a hypothesis a change in the duration of the action potential could result from a) change in the slope of phase 2 b) change in the threshold potential of repolarization c) change in the level of potential at the beginning of the plateau

Since the above hypothesis was formulated we have been informed from time to time about work being conducted on skeletal muscle which pointed to the possibility that potassium permeability in that tissue is inversely proportional to the electrochemical gradient acting on K^+ (A brief report of this work, by R H Adrian presumably will appear soon in the Proceedings section of the *Journal of Physiology*) A similar hypothesis is developed below in terms of cardiac muscle It will be seen that it entirely incorporates the earlier hypothesis advanced above, but it should be emphasized that it is equally speculative

An Ionic Hypothesis for Repolarization

If it is assumed that P_K is inversely proportional to the driving forces which act upon K^+, a remarkably large number of the observed properties of phases 2 and 3 may be explained *The driving force* which acts upon K^+ is the sum of a concentration gradient and an *electric field* The concentration *gradient can be reversed only* by extreme elevation of K_o^+, under physiological *conditions* K_o^+ is always lower than K_i^+, and the driving force of the concentration gradient is outward The electric field actually reverses During phase 4 the field tends to hold K^+ inside balancing the effect of the

concentration gradient, whereas during phases 1 and 2 the electric field aids the concentration gradient in moving K^+ from the inside to the outside of the fiber Thus during phase 4 the driving force acting on K^+ is nearly zero, and according to the hypothesis P_K is high During phases 1 and 2 the driving force acting on K^+ is high P_K therefore would be low

Under normal conditions such a property of P_K would produce repolarization in the following manner At or shortly after the end of phase 0, P_K would be low The gradual return toward E_K during phase 2 (which might result from an increase in K_o^+ immediately outside the fiber or from any of a number of other factors) would gradually increase P_K The interaction between P_K and transmembrane potential would serve to produce a regenerative increase in P_K during phase 3, which would account for the phase of rapid repolarization

Various special conditions can also be explained in terms of this hypothesis The action of anodal stimuli in inducing all-or none repolarization can be explained in terms of a shift in transmembrane potential towards E_K causing a rise in P_K The repolarization which results secondary to a rise in K^+ during phase 2 (Weidmann's 'K+ slug') would come about from the fact that an increase in K_o^+ increases P_K The numerous observations on the effects of acetylcholine hypoxia metabolic inhibitors and the like in abolishing the plateau are of course readily explained by the assumption that these various agents produce a more or less nonspecific elevation of P_K It is interesting to note that in theory a perfectly good action potential can be obtained in a fiber which has a high but unchanging permeability to K^+ and which merely undergoes a cyclic change in permeability to Na^+ It seems quite possible that the action potential of atrial fibers subjected to rather large concentrations of acetylcholine occurs in this manner This interpretation accords with the observation that under these conditions repolarization is very rapid that K^+ permeability is high and that the action potential amplitude is reduced It is this final point which suggests that K^+ permeability may be steadily high in such fibers

The failure of P_K to rise during prolonged depolarization under a voltage 'clamp may be interpreted simply P_K does not rise as the result of the passage of time but as the result of an elevation in K_o^+ or of a movement of the transmembrane potential toward E_K The

fact that a normal phase 3 appears after the release of such a "clamp" appears to agree with this interpretation It is also evident that rate-induced shortening may be interpreted in terms of an increase in P_K Whether this increase could be explained solely as the result of an elevation in K^+ is not clear, but probably some other factor is involved We may examine a summary of the observations which the theory might explain

1 In the presence of Na^+ carrier inactivation both P_N and P_K would be low during phase 2, and the transmembrane potential would be near zero R_m would be high during phase 2, since P_K would be low

2 Any accumulation of K^+ outside the fiber would lower the concentration gradient and thereby diminish the driving force and increase P_K which would accelerate repolarization

3 Accumulation of K^+ outside the fiber might very well occur as a result of increased rate If so, the observed effect of rate on action potential duration would follow

4 Artificial increase of K^+ either by increasing the concentration in the solution or by the injection of a "slug" of K^+ would similarly decrease the driving force, increase the permeability, and shorten the action potential

5 The sequence of phases of repolarization might follow from this theory If phase 1 represents early Na^+ inactivation phase 2 might well represent a slow rise in K^+ which slowly shifts P_K and E_m until the process becomes more or less regenerative and the more rapid phase 3 results

6 The observation that repolarization seems to be regenerative would fit with the idea that as E_m moves towards the resting potential, P_K increases and results in a further shift toward the resting potential

7 The same argument would explain why anodal stimuli induce sustained repolarization Such stimuli, if long enough and strong enough would result in a shift of E_m in a direction which would increase P_K

8 The retardation of repolarization by cathodal pulses similarly would result from the fact that holding the membrane at lower potential would hold P_K at a low level

9 The observation that action potentials of low amplitude also are short in duration would fit the theory since an action potential

of low amplitude would represent a smaller depolarization and therefore a lesser reduction of P_K

Two important agents Na+ and temperature, have not been mentioned Lowering temperature prolongs action potentials of cardiac fibers largely by increasing the duration of phase 2 Lowering Na+ shortens the action potential by shortening phase 2 Both these observations may be explained on the basis of the hypothesis outlined above In the case of low extracellular Na+ active inward transport of K+ might be decreased (Swan, personal communication) and thus K_o^+ would increase more rapidly, P_K would increase more rapidly and E_m would increase more rapidly In the case of low temperature the rate of active intrusion of K+ would be decreased which would cause a shorter action potential If on the other hand K+ efflux during phase 2 is diminished by a reduction in temperature the observed lengthening could be explained Such an explanation would require that R_m during phase 2 be increased by cooling It is not known whether this happens

It is interesting to note that this theory about the nature of P_K raises questions about pacemaker activity somewhat different from those advanced in Chap 5 If spontaneous depolarization occurs during phase 4 by any mechanism at all then the depolarization itself would diminish P_K thereby increasing R_m A reinterpretation of the idea that an increase in R_m indicates a decrease in P_K as the primary event in pacemaker activity might be required since any thing which led to depolarization would diminish P_K and increase R_m

It is apparent that the assumption that P_K is inversely proportional to the driving force acting on K+ can explain a great deal We lack demonstrative evidence for the theory and such evidence can be obtained only by membrane-impedance and ion flux studies of a type difficult to execute and interpret The observed fact that K+ leaves the fiber during activity does not necessarily imply that K+ permeability rises as has been pointed out elsewhere Finally, the *ad hoc* nature of the interpretation should be emphasized The same changes which are postulated to increase P_K might also be assumed to alter either P_N or the activity of a Na+ pump It is probably well to emphasize that no theory of repolarization has been advanced which has met the test of making radically new and correct predictions and thus uncovering new fundamental experimental knowledge In spite of this the theory given above seems promising

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The book is well illustrated with actual records from the clinic and laboratory It is the first work to comprehensively survey this important field The authors present the basic electrophysiologic explanations of clinical techniques and findings

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